The Ecology and Epidemiology of Devil Facial Tumour Disease

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Preface & Declaration by Author

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgment is made in the text of this thesis.

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Statement of Co-Authorship

Publications produced as part of this thesis:

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The following people and institutions contributed to the publication of research undertaken as part of this thesis:

Rodrigo Hamede: Contributed to ideas and study design, undertook fieldwork for data collection, carried out analysis and wrote the manuscripts.

Menna Jones: Contributed to ideas and study design, assisted with data analysis and edited the manuscripts.

Hamish McCallum: Contributed to ideas and study design, assisted with data analysis and edited the manuscripts.

Jim Bashford: Contributed to ideas and assisted with data analysis

Kathy Belov, Greg Woods, Shelly Lachish, Anne-Maree Pearse, Alex Kreiss, and Billy Lazenby: Contributed to ideas and interpreting results

We, the undersigned agree with the above stated “proportion of work undertaken” for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis.

Menna Jones	Erik Wapstra
(Candidate’s supervisor)	(Head of School)

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Abstract

Emerging infectious diseases are increasingly recognised as a significant threatening process in conservation biology. Empirical studies aimed at understanding the epidemiological and ecological processes underlying disease transmission and its impact on host populations are crucial for designing disease management strategies.

The Tasmanian devil (*Sarcophilus harrisii*), is threatened with extinction by Devil Facial Tumour Disease (DFTD), a novel and fatal transmissible cancer. In this thesis, I use proximity-sensing radio collars to reveal empirical contact networks in a wild devil population and infer the role of seasonal and demographic network structure dynamics in the epidemiology of DFTD. I further use this information to build disease-simulation network models to assess the role of contact heterogeneities in epidemic behaviour. Finally, I use longitudinal data sets that followed the natural progression of DFTD to compare contact patterns, epidemiology and impact of the disease in subpopulations which differ in their genotypic structure and diversity.

The results indicate increased frequency and length of male-female contacts during the mating season, compared with the non-mating season. These strong inter-sex contact preferences during the mating season suggest that the transmission dynamics of DFTD might be frequency dependent. There are strongly connected individuals in the network, but their identity changes with season, providing limited scope for targeting disease control actions to particular demographic groups.

Incorporating network structure and its seasonal and demographic dynamics in disease simulation models had a modest effect on the epidemic threshold for DFTD compared to traditional compartmental disease models. Quantifying heterogeneities in contact patterns and assessing their role in disease spread do, nonetheless, represent an important step for predicting epidemic behaviour and developing approaches for managing wildlife diseases.

This study has provided the first evidence of reduced impact and slow progression of DFTD in a wild population as the disease moves to a genetic subpopulation with major histocompatibility complex genes differing from those of the tumour itself. Biting patterns associated with disease transmission did not significantly differ between the two genetic subpopulations. Therefore, differences in genetic diversity in the host immune system or reduced virulence in the pathogen are proposed as plausible explanations for the epidemiological differences between subpopulations. In addition, I found that most tumours were located inside the oral cavity and devils with fewer bites were more likely to develop DFTD, which suggests that the probability of acquiring infection is higher in devils delivering bites than in those receiving bites.

The findings of this study, which examined the natural progression of an emerging disease in an ecological and epidemiological context, have direct implications for designing future conservation strategies for the species, and are broadly applicable to a range of other conservation challenges posed by wildlife diseases.

Keywords: Tasmanian devil facial tumour disease**,** wildlife disease ecology, social networks, contact rates, epidemiology, infectious cancer, disease transmission, social behaviour, seasonality, biting patterns, disease management.

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CHAPTER 1

General Introduction

The ecological and evolutionary context of infectious diseases

The role of infectious diseases in human populations is well-established and is becoming increasingly important as human population densities and their interactions with wildlife and livestock increase. However, until the seminal works of Anderson and May (1979) and May and Anderson (1979), the importance of infectious disease as a driver of the dynamics of nonhuman populations was somewhat neglected in ecology. Anderson and May's (1979) work enabled the population biology of infectious diseases to be understood from ecological, quantitative and mathematical perspectives. In more recent years, this discipline has come to maturity with a consensus that host-pathogen ecology should be unified with other research disciplines such as behavioural ecology, mathematical modeling, epidemiology and veterinary medicine. Wildlife epidemiology has recently emerged as an essential and multidisciplinary field within biodiversity conservation and human health (Daszak et al. 2000; Lafferty and Gerber 2002; Daszak and Cunningham 2003).

Interactions between hosts and infectious diseases can be understood only in the light of coevolutionary processes. Whilst some studies suggest that host pathogen interactions are unlikely to evolve towards complete commensalism (e.g. May and Anderson 1983), the impact of pathogens on their host populations is particularly severe when the interaction is new in evolutionary terms. The introduction of exotic virulent pathogens into naïve populations can result in epidemic outbreaks with high mortality. The measles and smallpox pandemic in the Americas caused by the Spanish conquistadors during the 1500's (McNeill 1976) and the Rinderpest panzootic in African herbivores after Europeans introduced cattle in the African continent (Plowright 1982), are

prominent examples of large scale and catastrophic epidemics resulting from the introduction of pathogens into naïve populations.

During the last three decades humans have been affected by an unprecedented number of emerging infectious diseases such as hantavirus pulmonary syndrome, Lyme disease, severe acute respiratory syndrome (SARS), avian influenza and many others, all of which are classified as zoonoses, transmitted to humans from natural reservoir wildlife species. At the same time, a large number of wildlife species have been affected by pathogens which spilled over from domestic species (see Daszak et al. 2000). Anthropogenic environmental changes are recognised as a cause for the emergence of wildlife diseases, which pose threats to human health, domestic species and biodiversity (Daszak and Cunningham 2003). Diseases can also emerge as natural processes within ecosystems. Several studies have recognised evolutionary and ecological processes as important drivers of disease emergence (Daszak et al. 2000; Boots and Sasaki 2003; Woolhouse et al. 2005; Jones et al. 2008b). While ecological drivers for disease emergence have been more frequently associated with changes in host-pathogen ecology such as increase in population density and contact rates, environmental changes and changes in host mobility or behaviour, evolutionary drivers for disease emergence include high genetic variability in pathogen populations or low genetic variability in host populations (Daszack et al. 2000; Altizer et al. 2003; Woolhouse et al. 2005; Pedersen and Davies 2009).

Infectious diseases can occur in two forms, as endemic or epidemic infections. Endemic infections are those which persist in a host population without extensive fluctuations in prevalence or dramatic effects on the host population, tending to cause host morbidity

rather than mortality (Moon and Gould 2000). Conversely, epidemic infections are usually characterized by a rapid increase in prevalence, followed by a rapid decrease in prevalence as the number of susceptible hosts is exhausted (Moon and Gould 2000). Following an epidemic, the pathogen may disappear entirely from the local host population or may decline to a low level. Epidemics are often, but not necessarily, associated with high mortality. Usually, pathogens spreading into naïve populations - within and across species - initially cause major epidemics with the potential to lead to major population declines (Robinson et al. 2010).

Infectious diseases are an important part of ecosystems, and their dynamics and effect on wild populations are an essential part of ecosystem adaptability and evolution. Pathogens play vital roles in natural ecosystems, such as regulating population dynamics (Dobson and Meagher 1996), altering host genetic diversity (Duffy and Sivars-Becker 2007) or shaping their functional adaptability and coevolutionary fitness (Boots and Sasaki 2003). With increasing interaction between humans and wildlife and declining and fragmented wildlife populations, however, wildlife diseases have been increasingly recognised as a key factor involved in local extinction and threatening processes (Haydon et al 2002; Smith et al. 2006; Pedersen et al. 2007). Pathogens have become a major concern in conservation biology because they can trigger or accelerate population declines in species that are already subject to other endangering processes (eg. Daoust et al. 2009) or previously thriving populations (eg. Kennedy et al. 2000). The regulation, dynamics and impact of wildlife epidemics on host populations can also have direct or indirect consequences at community and ecosystem scales (Tompkins et al. 2011).

The particular concern of infectious disease for keystone predators

Predators play a key ecological role in structuring communities and in protecting biodiversity, and their loss can have a broad range of cascade effects in the ecosystem (Smith et al. 2003; Glen et al. 2007; Dickman et al. 2009). Predators are also more likely to be at risk of extinction than other taxa and virulent pathogens play a central role in their ecology and conservation (Pedersen et al. 2007). Trophic level influences the impacts of disease on wildlife populations. Wildlife diseases are considered to be of particular concern for carnivores given that many populations, particularly of terrestrial carnivores, are highly susceptible to and already subject to endangering processes, such as habitat destruction and/or fragmentation, overexploitation of their prey or direct persecution, and that they have an important trophic role in ecosystems (Funk et al. 2005). Significant population declines in carnivores that have been attributed to disease encompass a broad range of taxa (see Table 1).

Several studies have shown that one of the most significant risk factors for keystone predators is spillover of infectious diseases from domestic animals. For example, canine distemper virus (CDV) and rabies have been responsible for dramatic population declines in critically endangered species such as African wild dogs (*Lycaon pictus*) (Alexander and Appel 1994; van de Bildt et al. 2002), Ethiopian wolves (*Canis simensis*) (Sillero-Zubiri et al. 1996; Randall et al. 2004) and black footed ferrets (*Mustela nigripes*) (Williams et al. 1988) as well as sudden mortalities in Canadian lynx (*Lynx canadiensis*) (Daoust et al. 2009). Similarly, canine parvovirus has caused long term demographic effects on grey wolves (*Canis lupus*) (Mech and Goyal 1995). Spillover is a consequence of human encroachment into wildlife habitat, which has

increased contact between wild and domestic animals, bringing previously isolated species and their pathogens into more frequent contact. Infectious pathogens may have severe consequences for threatened species, both directly and through reservoir hosts in domestic animals. Anthropogenic impacts are a common theme among the several factors associated with the recent spread of wildlife diseases (Daszak and Cunningham 2003). The transmission and spread of disease from domesticated species to sympatric wildlife has nowadays become a serious problem for carnivore species of conservation concern.

While there is awareness of the rising number of catastrophic epidemic outbreaks in wildlife, the detailed and long-term studies necessary to understand and manage these diseases for conservation are often lacking. The effects of disease and its transmission dynamics are often complex, especially when the decline in host populations cause trophic cascade effects (Holdo et al. 2009) or for diseases with multiple reservoir hosts as these can maintain the pathogen at a high force of infection, even when the affected population declines towards extinction (de Castro and Bolker 2005). Understanding the mechanisms for disease invasion and persistence requires a detailed knowledge of the ecology and behaviour of both hosts and pathogens. Such information is rarely available for many host-pathogen systems, particularly in wildlife. There is a large gap between theoretical research and empirical data on how wildlife diseases affect carnivores. For example, there is little evidence from empirical studies on how parasites and diseases can regulate carnivore communities at the ecosystem level (but see Lembo et al. 2008). Likewise, for many infectious diseases determining the extent to which disease induced mortality leads to compensatory responses requires long-term epidemiological studies,

which are often impractical or economically unviable. There is a clear need to study and apply epidemiological studies in carnivore conservation.

Table 1.1 Infectious diseases in carnivores that have resulted in large population declines or local extinctions

Family	Species	Pathogen	Reference
Canidae	African wild dog ^ (*Licaon pictus*)	Canine Distemper Virus	Alexander and Appel 1994
		Rabies	Gascoyne et al. 1993
	Ethiopian wolf * (*Canis simensis*)	Rabies	Randall et al. 2004
	Gray fox * (*Urocyon cinereoargenteus*)	Canine Distemper Virus	Davidson et al. 1992
	Island fox * (*Urocyon littoralis*)	Canine Distemper Virus	Timm et al. 2009
Mustelidae	Black-footed ferret ^ (*Mustela nigripes*)	Canine Distemper Virus	Williams et al. 1988
	Souther sea otter * (*Enhydra lutris*)	Protozoan parasite (*Sarcocystis neurona*)	Miller et al. 2010
Felidae	African lion * (*Panthera leo*)	Canine Distemper Virus	Roelke-Parker et al. 1996
	Iberian lynx * (*Lynx pardinus*)	Feline leukaemia	Lopez et al. 2009
	Canadian lynx * (*Lynx canadiensis*)	Canine Distemper Virus	Daoust et al
Phocidae	Harbour seal * (*Phoca vitulina*)	Phocine Distemper Virus	Harkonen et al. 2006
	Siberian seal * (*Pusa sibirica*)	Canine Distemper Virus	Grachev et al. 1989
	Crab eater-seal * (*Lobodon carcinophaga*)	Canine Distemper Virus	Barrett 1999
Dasyuridae	Tasmanian devil * (*Sarcophilus harrisii*)	Devil Facial Tumour Disease	Hawkins et al. 2006

* indicate population decline, ^ indicate local extinction

Ecological interactions of wildlife diseases

Transmission dynamics, R_0 and population thresholds

Key concepts in disease ecology are the basic reproductive number (R_0) (Hesterbeek 2002) of the disease and the host population threshold (Lloyd-Smith et al. 2005a). Theoretically, the establishment and maintenance of any pathogen is related to R_0, the expected number of secondary cases generated by a primary case in a wholly susceptible population. If $R_0 < 1$ each infected case cannot replace itself and the disease will die out, but if $R_0 > 1$ an epidemic outbreak may occur. The host population threshold is the minimum population density required for disease invasion and persistence (Lloyd-Smith et al. 2005a). The concept of host population threshold is widely used as a framework for designing disease control strategies based on culling (Caley et al. 1999), sterilization (Caley and Ramsey 2001) or vaccination (Tompkins et al. 2009). Estimating clear-cut population thresholds for wildlife diseases, however, is usually hampered by practical constraints such as limited or biased data sets and lack of replication (Lloyd-Smith et al. 2005a).

The transmission dynamics of wildlife diseases are categorized into two classic models, density dependent and frequency dependent transmission, although these two modes represent two ends of a continuum of transmission modes (Smith et al. 2009). Density-dependent transmission (usually represented as $ßSI$, where $ß$ is the transmission coefficient, and S and I are the density of susceptible and infected hosts respectively), is when the risk of infection is directly scaled with the density of infectious individuals. In this scenario, increasing host density leads to an increase in the number of contacts between hosts and thus the number of transmission events. Frequency-dependent

transmission (usually represented as *ßSI/N*, where *N* is the total population size) is when transmission scales with the proportion of infectious individuals. Here, the number of contacts remains constant with increasing host density and, therefore, it is the proportion of potentially infectious contacts that determines the number of transmission events. However, determining the transmission mode that is actually operating in a given host-pathogen system is particularly difficult and has been a matter of a long and active debate (de Jong et al. 1995; McCallum et al 2001; Begon et al. 2002). For a simple susceptible-exposed-recovered model under the density-dependent scenario R_0 can be calculated as:

$$R_0 = \frac{\beta N}{a + b + v}$$

where a is the per capita disease-induced mortality rate, b is the per capita natural mortality rate and v is the recovery rate from disease. Here, β is the transmission rate per unit of time. The threshold host density (N_T) for disease invasion can easily be obtained by setting R_0 to 1 in the above equation, yielding

$$N_T = \frac{a + b + v}{\beta}$$

For the frequency-dependent scenario R_0 can be calculated as:

$$R_0 = \frac{\beta}{a + b + v}$$

here the transmission rate ß is independent of population size. In this case, there is no threshold host density for disease invasion. Because frequency dependent diseases (eg. sexually transmitted diseases or transmitted by vectors) lack a threshold population density for which the pathogen cannot be maintained, they are more likely to cause disease –induced extinctions (de Castro and Bolker 2005).

In principle, there are four possible alternatives by which R_0 can be brought below its threshold ($R_0 < 1$):

- First, by reducing the transmission rate β. This is more achievable in human than in wildlife diseases because humans can be questioned and directed. In human populations, potential infectious contacts can be reduced by several mechanisms, such as quarantining infected individuals, developing patient contact databases for tracing of infectious contacts, and developing strict control policies aimed at reducing the intimacy and frequency of social interactions within a population. Employing such strategies in wildlife populations is not entirely impossible, but is obviously more difficult than in human populations. Wobeser (2002) suggests that habitat modification to reduce transmission may be a viable means of managing wildlife disease. As β is a product of both the contact rate and the probability of disease establishment following contact, vaccination can be thought of as a means of reducing the transmission rate. While vaccinations are available for many human diseases, vaccination coverage at a scale necessary to eradicate or even control disease outbreaks in wildlife populations is, in many cases, impracticable (but see Smith and Dickson 2003; Sidwa et al. 2005).
- Second, by increasing the recovery rate from infections, provided there is a disease treatment available. Again, this is far more likely to result as an effective disease control strategy in human populations as treatment for disease can be delivered in practise to most, if not all of the population in question. For wildlife diseases, few treatments are available and even fewer can be delivered effectively to wild populations.
- Third, by increasing the disease-induced mortality rate (eg. culling infected individuals), which will also have the effect of reducing transmission.

Controlling disease outbreaks by selective culling operates on the basis of reducing R_0 below one, which in theory, will lead to the extinction or elimination of the disease. This is only applicable to wildlife or livestock diseases and although is has been widely used in many host-pathogen systems (Caley et al. 1999; Jenkins et al. 2010, Lachish et al. 2010) its overall effectiveness is in many cases hampered by the interaction of ecological effects (Woodroffe et al. 2009).

- Fourth and finally, by culling both infected and uninfected hosts. An extension of this concept is "stamping out", whereby all individuals in the affected area are removed. Indiscriminate culling can affect R_0 in two ways. It can decrease population size, applicable only for diseases with density-dependent transmission. Second, it increases the disease-independent death rate (b in the equations above). This strategy is used in highly infectious diseases in livestock that have a very high R_0, such as foot-and-mouth disease (Tildesley et al. 2009). Indiscriminate culling, and in particular the removal of all individuals in a population as in "stamping out", is often considered unacceptable for threatened wildlife or critically endangered species.

The presence of reservoir hosts or inter-species transmission is another critical epidemiological aspect which affects the ability to control or eradicate an infectious disease from a population. There are many examples of wildlife diseases spreading from one host species within which the infection is endemic or has low mortality or virulence to other species where the infection is epidemic. These cases are well-illustrated by badgers (*Meles meles*) and brush-tailed possums (*Trichosurus vulpecula*) which maintain and spread Bovine tuberculosis (Tb) infection in cattle (Woodroffe et al. 2005;

Coleman et al. 2006) and by domestic dogs which are responsible for the spillover and spread of rabies to several carnivore species within African ecosystems (Lembo et al. 2008). Furthermore, most infectious diseases currently threatening wildlife species with extinction are maintained by one or more reservoir species (van Riper 1986; Lips et al. 2006; Randall et al. 2006; Vial et al. 2006). This means that the pathogen can be maintained even when affected populations are at extremely low densities and even when they are brought towards extinction. The difficulties of eradicating diseases with multiple hosts have remained as one of the most important challenges for wildlife epidemiologists and conservation groups.

The role of contact heterogeneities and network theory in the epidemiology of infectious diseases

Transmission is the principal force driving the dynamics of infectious diseases. Thus, the crucial aspect that needs to be parameterized to understand the transmission dynamics of a given disease is the rate at which susceptible hosts acquire infection as a result of their contact with infectious hosts. The traditional starting point of epidemiological studies is to assume random interactions between individuals in a population. More precisely, the assumption is that estimating the mean rate of contact between individuals is sufficient to understand transmission. This is known as a "mean field" assumption. However, social relationships and contact rates within a population are neither random nor homogeneous. Individuals often form preferential associations and interact on the basis of social assortativity and hierarchical structure (Krause and Ruxton 2002) as well as in response to other ecological factors such as density dependence (Barlow 1996) or seasonal dynamics (Hosseini et al. 2004). These heterogeneities in host contact patterns can profoundly affect the dynamics of disease

transmission. In recent years, the use of Social Network Analysis (SNA) (Wasserman and Faust 1994) has provided a major step towards better understanding the effect of contact heterogeneities in the transmission dynamics and spread of infectious diseases (Meyers et al. 2005; Bansal et al. 2007). SNA arose as a major field in modern sociology and its uses were quickly applied to other disciplines such as communication studies, economics, information science, biology and epidemiology. Network theory views the social relationship in terms of 'nodes' (individuals within a network) and 'edges' (social contacts or relationships between individuals) (Waserman and Faust 1994). Epidemiological studies using SNA often look at global statistical properties of network data and provide a qualitative and quantitative framework that can be used to characterise the social interactions at individual, population or metapopulation levels. From a network perspective, the probability of an individual acquiring infection is a function of how many connections it has in the network, the frequency and intensity of those connections and the proportion of infected neighbours.

The structure of a contact network plays a critical role in the transmission dynamics of infectious diseases (Newman 2002, 2003; Keeling 2005; Meyers et al. 2005). For example, some networks are characterised by having a small proportion of individuals with anomalously high numbers of infectious contacts (which are termed "superspreaders") (Lloyd-Smith et al. 2005b) and will therefore have important epidemiological implications. These types of networks are called "scale-free networks" (Barabasi and Albert 1999), because the number of contacts of individuals within the network follows a power law distribution. Unlike bell-shaped distributions, power law distribution does not have a peak and so the frequency of contacts has a long tail

characterized by a continuous decreasing function. Effectively, this means that scale-free networks can have very low or no epidemic threshold.

Other important structural properties of networks relevant to the spread of infectious pathogens are the connectivity and clustering of individuals. Watts and Strogatz (1998) defined “small-world networks” as those characterized by high levels of local clustering and global connectivity. In this case, most individuals are not connected to each other but most of them can be reached from every other by their neighbours in common. In small-world networks the typical distance or the number of steps required to connect two individuals grows proportionally to the logarithm of the number of individuals in the network. The functional significance of small world networks is that because of their high connectivity, disease spreads faster than in random networks (Watts and Strogatz 1998).

Characterizing network structure has become a multidisciplinary research focus, which has been useful in understanding the processes underlying disease transmission and to predict their epidemiological behaviour. Network theory has been applied in the design of control strategies in human diseases such as SARS (Anderson et al. 2004; Meyers et al. 2005), HIV and other sexually transmitted diseases (Liljeros et al. 2001; Galvani and May 2005; Perisse et al. 2010), and avian influenza (Bansal et al. 2006). Despite the extensive use of network theory in human epidemiology, its applications in wildlife studies are preliminary (see, for example McCallum 2009; Perkins et al. 2009). The lack of high resolution data on wildlife interactions that are needed to unravel the behavioural processes that affect contact rates and disease transmission have been the major limitations for using network theory in wildlife epidemiology. However, recent

technological advances in telemetry may improve the scope for animal surveys and refine the data collection process (Prange et al. 2006; Krause et al. 2011).

The application of network theory to wildlife disease epidemiology has enormous potential (May 2006). First, it can be used to greatly refine the estimation and parameterization of the frequency, duration and intensity of interactions between individuals in a population. Second, it can be used to identify socially central individuals or demographic groups which may be more influential for disease transmission and assess their role in the network structure (Bohm et al. 2009). We know relatively little about how individuals operate within existing social structures and to what extent that affects their contact rates. SNA provides a deeper understanding of social complexity and population structure and can therefore integrate individual animal behaviour with epidemiology and population biology. Third, using estimations of network structure, epidemiological models can be used to predict the pathway and speed of disease spread. Most epidemiological studies are retrospective, providing limited potential to control disease outbreaks. The use of network theory in preventative veterinary medicine has been critical for estimating potential risks of disease spread in farm animals, which can then spill over to wildlife populations (Keeling et al. 2003; Dube et al.; 2008; Kiss et al. 2008; Martinez-Lopez et al. 2009). In addition, disease simulation outbreaks in network models can be used to examine basic assumptions of disease-causing contacts, evaluating network structural properties relevant to disease transmission and assessing the efficacy of potential control measures (Witten and Poulter 2007; Martinez–Lopez et al. 2010).

Finally, experimental manipulation of networks can assist in addressing important epidemiological questions such as the effect of population structure in disease spread, the types of interactions that favour disease transmission or the role of certain individuals in disease persistence. For example, Flack et al. (2006) used network theory to characterize social structure in pigtailed macaques (*Macaca nemestrina*). A controlled experiment demonstrated that the presence of certain highly-ranked individuals was necessary to maintain group cohesion and that the removal of these individuals resulted in the network breaking into several sub-units due to increased conflicts within the remaining individuals. Similarly, Corner et al. (2003), followed transmission of bovine tuberculosis in a captive population of brushtailed possums (*Trichosurus vulpecula*) and found that infected individuals had greater scores of clustering and connectivity than those that remained free of infection. The authors also found that changing the physical environment of the enclosures either by changing the number of dens or relocating individuals to different enclosures did not affect the social structure of different study groups. Both studies highlight the importance of using network theory for understanding the effect of population structural properties in the transmission dynamics of pathogens, predicting epidemic behaviour and for developing disease control strategies.

Disease tolerance, resistance and susceptibility

Infectious diseases are ubiquitous and play an important role in driving the dynamics and evolution of wild populations (Weatherall 2003). Following exposure to a pathogen, a range of outcomes can result at the individual and population level, from differing levels of infection intensity to adaptive responses including disease resistance or disease tolerance. While disease resistance is the ability to limit or prevent infection after being

exposed to a pathogen, tolerance is the ability to limit or prevent the health or fitness consequences of infection after exposure (Raberg et al. 2009). The critical difference between these two host responses is that from an ecological and evolutionary perspective resistance confers protection to the host at the expense of the pathogen whereas tolerance saves the host from the effects of infection without having direct negative effects on the pathogen (Raber et al. 2009). Thus, resistance and tolerance are expected to have different coevolutionary implications as well as differing epidemiological and ecological consequences (Boots 2008; Carval and Ferreire 2010). For example, resistance has a negative effect on pathogen fitness and therefore is expected to reduce the prevalence in the host population. Tolerance on the other hand, is expected to have a positive effect on the pathogen since as hosts live longer when infected, tolerant hosts should increase the infectious period of the pathogen (Roy and Kirchner 2000; Miller et al. 2006). Although most of the empirical evidence of the effects of tolerance and resistance in ecological, epidemiological and evolutionary processes comes from plant biology and the agriculture, there has been an increasing number of studies applying the statistical and evolutionary framework of tolerance and resistance to animal populations (Raberg et al. 2007, 2009; Carval and Ferreire 2010).

Trade-offs for the host are likely to exist between investment in immune defence and infection. Such trade-offs will depend on the long-term effects of infection on fitness, reproduction, life-history and survival. Several empirical studies provide good examples of these trade-offs. Telfer et al. (2005) found that woodland rodents affected with cowpox virus were more likely to delay breeding until the following year than uninfected individuals of the same species. Conversely, Jones et al. (2008a) discovered early sexual maturity and precocial breeding in Tasmanian devil (*Sarcophilus harrisii*)

populations affected by Devil Facial Tumour Disease (DFTD), a lethal infectious cancer that causes greater adult than juvenile mortality, and Schwanz (2008) demonstrated that deer mice (*Peromyscus maniculatus*) affected by chronic parasitic infection had litters with heavier mass than uninfected mice. Even when disease does not directly affect host life history strategies, reproductive success or survival, the costs of immune-associated responses can influence host susceptibility to other infections (Beldomenico and Begon 2010).

Susceptibility to infection of hosts can be related to body condition, with individuals in poor condition being more likely to have compromised immune systems. These individuals could be more likely to become infected and more important in driving the dynamics of infection within a population. In natural populations, susceptibility to infection and host condition might act in synergy, triggering vicious cycles in which poor condition predisposes higher risk of infection which further reduces host condition and increases infection intensity (Beldomenico and Begon 2010). Such is the case of field voles (*Microtus agrestis*) affected by a specific and naturally occurring protozoan, *Trypanosoma microti*, in which the intensity of infection was dependent on the preceding health status, measured by red blood cells and lymphocyte counts (Beldomenico et al. 2009). In addition, the study concluded that the effect of the pathogen on host condition was dependent on infection intensity. Likewise a study from Blanchet et al. (2009) found that the extent of parasitic infection with copepods in the rostrum dace fish (*Leuciscus leuciscus*) was associated with the growth rate of the fish before infection. The study also concluded that the burden of parasites and subsequent degradation of fins was better explained by models considering growth rate as a cause rather than as a consequence of parasitic infection.

Loss of genetic variation at the Major Histocompatibility Complex (MHC), a complex of genes which is critical for generating immune responses in vertebrates, may result in decreased immune and adaptive responses against pathogens (Spielman et al. 2004; Radwan et al. 2010; Belov et al. 2010). For small or inbred populations where MHC heterozygosity is lost, deleterious recessive alleles may become more common or fixed due to genetic drift (Lande 1994). Roelke et al. (1993) found that the critically endangered Florida panther (*Felis concolor coryi*), reduced to less than 50 individuals, was more susceptible to microbial parasites than the larger populations of panthers in western United States. Several other species that have experienced severe population bottlenecks have been shown to have reduced variation at the MHC, eg. cheetah (O'Brien et al. 1985), Eurasian beaver (Babik et al. 2005), giant panda (Zhu et al. 2007) and European bison (Radwan et al. 2007). Although MHC host genetic diversity plays an important role in buffering populations against pathogens, there is limited rigorous evidence correlating loss of genetic diversity affecting the long-term viability of host populations (Acevedo-Whitehouse and Cunningham 2006; Radwan et al. 2010). Clearly, further empirical studies are needed to test associations and causality between MHC diversity and disease resistance or susceptibility (Spielman et al. 2004).

Differences among hosts in susceptibility, resistance and tolerance to infection are important for interpreting the dynamics of wildlife diseases. These aspects are, however, assumed to be invariable in most studies of disease ecology and in most epidemiological models. For many wildlife populations, the average susceptibility and variation in susceptibility are dynamic, and depend on a range of factors including body condition, stress, availability of resources, phenotypic differences, exposure to environmental

toxins and immuno-genetic diversity. Inclusion of variation in host susceptibility, resistance and tolerance to disease in epidemiological studies might offer new insights into the causes and consequences in host-pathogen interactions, the effect of disease in predator-prey systems (Hatcher et al. 2006) and host-pathogen co-evolutionary processes (Lambrechts et al. 2006; Carval and Ferreire 2010). Understanding how pathogens have shaped the genetic make-up and disease resilience of host populations is also vital for designing effective treatments and vaccines.

Managing infectious diseases in wildlife: applications and future challenges

Determining the ecological impact of a disease in host populations is necessary for the implementation of effective management strategies. However, disease is a common and dynamic feature of wildlife populations and establishing its overall impact requires robust and long-term datasets. As discussed above, some diseases may have a direct impact on host survival, others might affect host reproduction, whereas some pathogens with low morbidity might not directly affect either survival or reproduction. In addition to the pathogenicity of a disease and the host adaptive response, other ecological factors such as density or frequency dependent effects or the existence of host reservoir species can significantly alter the impact of a disease. Seasonal, demographic or behavioural cues might also interact in synergy with the dynamics of disease on host populations (Altizer et al. 2004; Hosseini et al. 2004; Kiesecker et al. 1999). Therefore, establishing the extent to which an infectious disease threatens a population and assessing the required management strategies to mitigate its effect is a complex task (Haydon et al. 2002).

Mathematical modelling has become one of the most important tools for predicting the spread and impact of wildlife diseases as it provides a bridge for integrating epidemiological and demographic data. The main purpose of modelling epidemics is to understand the processes by which an infectious agent spreads, so the efficiency of different control actions and management strategies can be assessed. However, mathematical models need accurate information on the nature and frequency of interactions to produce realistic outcomes. Providing this information has been one of the major challenges for wildlife epidemiologists (but see Krause et al. 2011). Unlike humans, which can participate in diary - based contact surveys (Read et al. 2008), and be subject to contact tracing (Eames and Keeling 2003), contact patterns in wildlife are difficult to establish and parameterise.

Available methods for intervention in wildlife diseases, that aim to either eradicate disease or lessen its impact, are few and are often difficult to implement in wild populations. Vaccines are available for some infectious diseases in wildlife, particularly for those caused by viruses that also infect domestic livestock or humans (Smith and Wilkinson 2003, Sidwa et al. 2005). Vaccination programs have been implemented to prevent the spread of diseases in several critically endangered species such as African wild dogs (Woodrofe 2001; Vial et al. 2006), Ethiopian wolves (Haydon et al. 2006; Knobel et al. 2008) and Iberian lynx (Lopez et al. 2009). However, as discussed above, vaccination programs are mainly used in preventing disease outbreaks rather than in eradicating disease once is established or in reducing its impacts on host populations. In addition, vaccination programs can have counterproductive effects in the populations in question. Woodroffe (1999) argued that vaccination programs against phocine

distemper virus in harbour seals (*Phoca vitulina*) were considered unsafe since the protocol required multiple dose administration and was regarded as too stressful for this marine mammal. The same study also argued that in some cases precluding hosts from infections through vaccination might alter the evolutionary processes involved in selective inheritance of disease resistance. Similarly, vaccination programs against rabies in African wild dogs were under a moratorium period after a conservation plan claimed that handling and vaccination stress could be an important factor related to local extinctions (Woodroffe et al. 1997). Finally, for many emerging infectious diseases (eg. DFTD [Woods et al. 2007] and chytridiomycosis [Kilpatrick et al. 2010]) there is no available vaccine or treatment.

Culling is a management strategy that is commonly used to manage wildlife populations affected by infectious disease (Wobeser 2002; Wassenberg et al. 2009; Jenkins et al. 2010). As discussed above, culling is expected to be more applicable as a management strategy for diseases that are strongly density dependent (sensu McCallum et al. 2001). A much greater level of removal of infected individuals or of the entire population is required to control a strongly frequency dependent disease (Wassenberg et al. 2009; Lachish et al. 2010). This strategy is commonly implemented with livestock diseases such as foot and mouth (Fergusson et al. 2001), but is not likely to be acceptable for a small or endangered wildlife population as it may increase extinction risk (McCallum and Jones 2006). When culling is used to reduce population density, the degree of population reduction necessary to eradicate a disease or to prevent its spread is often difficult to estimate (Wobeser 2002). Several studies testing the effect of culling European badgers (*Meles meles*) to reduce the incidence of bovine tuberculosis have suggested that the reduction in population density may be counterproductive as it

disturbs badger social organization and movement, ultimately increasing disease spread (Donelly et al. 2003, 2006; Pope et al. 2007; White et al. 2008). While culling programs are routinely and successfully used to control disease outbreaks in domestic species (eg. foot and mouth disease [Tildesley et al. 2009] and avian influenza [Suguira et al. 2009]) its application for endangered species or small populations needs to be carefully considered.

Surveillance and monitoring of disease outbreaks are essential for understanding the epizootiology of infectious diseases and designing effective management strategies (Morner et al. 2002; Wobeser 2002). A major limitation in the identification of emergent disease agents in wildlife is the lack of diagnostic capability for infectious diseases that do not affect humans or domestic animals. Because of a paucity of surveillance for emerging wildlife diseases (Morner et al 2002), these are not detected until the epidemic has caused significant impacts on host populations. Advances in diagnostic research and treatment as well as increased surveillance for wildlife diseases are urgently needed to enable a proactive approach towards detecting and managing diseases prior to sometimes disastrous conservation impacts of epidemics (Morner et al. 2002; Funk et al. 2005). Cross-disciplinary integration of field, laboratory and theoretical research should provide new insights into the broader ecological processes determining disease dynamics and their effects at the population and ecosystem levels.

The Tasmanian devil (*Sarcophilus harrisii*) and Devil Facial Tumour Disease

Endemic to Tasmania, devils were formerly distributed across mainland Australia until their extinction approximately 3000 to 4000 years ago, coinciding with the anthropogenic introduction of the dingo (*Canis lupus dingo*) (Archer and Baynes 1972; Corbett 1995; Brown 2006) and a rise in technology and population size of aboriginal peoples (Johnson and Wroe 2003). Tasmanian populations became separated from those of mainland approximately 12000 years ago at the end of the last glaciation when rising sea levels flooded the Bassian plains. Low genetic diversity in the current Tasmanian population is consistent with low diversity in this species at the time of continental separation or an island founder effect (Jones et al. 2004). After the extinction of the Thylacine (*Thylacinus cynocephalus*), the last documented individual of which died in 1936 (McKnight 2008), the Tasmanian devil remains the largest extant marsupial carnivore (*Dasyuridae*) and this former mesopredator is now the top predator in the island state of Tasmania.

Devils are nocturnal predators and specialized scavengers that occur in a broad range of habitat types, including dry sclerophyll forests, mixed wet forests, coastal woodlands, grassy woodlands and open grasslands (Jones and Rose 1996). Devils are sexually dimorphic: average male weight is 10kg while female weight averages 7kg. Like most dasyurids, devils have a short life-span, living for up to six years in the wild (Pemberton 1990; Jones et al. 2008a). The mating system of devils is promiscuous; males usually guard females in dens for several days and mate with several females during the mating season (Guiler 1970; Hesterman 2008). Devils are synchronized annual breeders, with most births occurring from early March to mid April (Hesterman et al. 2008). Females

usually achieve sexual maturity at the age of two years, but are capable of breeding at around 15 months of age if nutrition is good, and they become reproductively senescent in their fifth or sixth year (Jones et al. 2008a; Lachish et al 2009). Although females can produce a maximum of four young per litter the average number of young per breeding female is 2.8 (Pemberton 1990) and gestation period ranges from 14-22 days (Hesterman et al. 2008). Young become independent and disperse just prior to the next mating season, 9-10 months after birth. Devils are non-territorial with individuals having overlapping home ranges (average home range is 10-13 km^2) (Pemberton 1990). Whilst the devil is a solitary species, aggregations at food sources are common, where agonistic behaviour and biting activity are very frequent (Pemberton and Renouf 1993; Hamede et al. 2008).

Until the emergence of DFTD in 1996 in northeastern Tasmania, devils were regarded as common (Hawkins et al. 2006). DFTD has now spread southward and westward and has been confirmed in most of the distributional range of the devil (see Fig. 1.1). Spatial epidemiological models have estimated that it is probable that the disease will reach the devil's entire distributional range within ten years (McCallum et al. 2007). Significant population declines associated with DFTD have been reported in most populations where monitoring has been undertaken (McCallum et al. 2009). As a result the Tasmanian devil has been listed as endangered at State (Tasmanian Threatened Species Protection Act 1995; www.dpipwe.tas.gov.au) national (Australian Government Environment Protection and Biodiversity Conservation Act 1999, www.environment.gov.au/epbc) and international levels (IUCN threatened species list; www.iucnredlist.org).

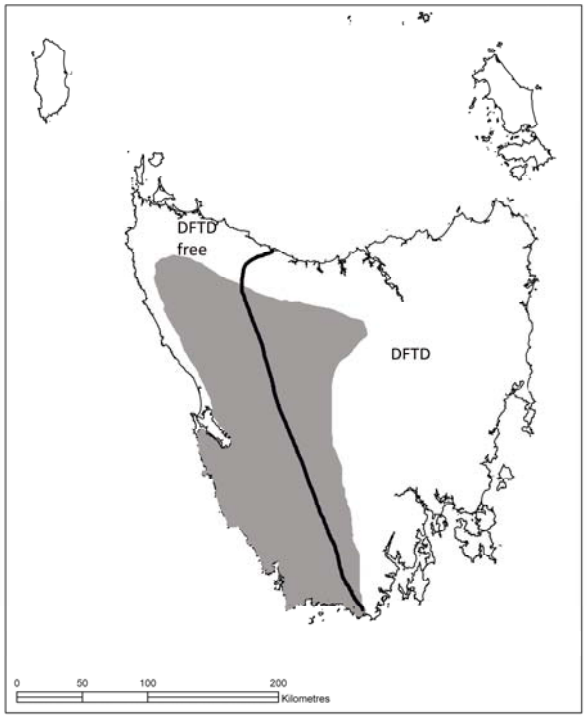

Figure 1.1 Map of Tasmania showing the distribution of DFTD (at May 2011). The grey shaded area represents low devil density due to unsuitable habitat (Jones and Rose 1996) and the black line separates DFTD and DFTD-free areas.

DFTD is an unusual infectious cancer in which the tumour cells themselves are the infectious agent. Most cancers are not infectious and are thus rarely associated with population declines or mass mortalities. In recent decades, however, several infectious cancers in wildlife have been reported from affecting endangered species (Table 1.2) and thus they are increasingly considered a conservation threat (McAloose and Newton 2009; McCallum and Jones, in press). Transmission of DFTD occurs in the form of an allograft, when individuals directly infect another individual with live tumour cells probably mostly through penetrating bites (Pearse and Swift 2006). The establishment of the tumour cell line is facilitated by extremely low genetic diversity (Jones et al. 2004), particularly at the MHC (Siddle et al. 2007), in which the recipient host fails to recognise the foreign tumour cells as nonself. Contagious cancers are a special case of

infectious disease in which low genetic diversity at the MHC appears to be important. The transmission of live tumour cells from infected to susceptible individuals distinguishes directly transmissible cancers from those that have a viral origin in which the virus rather than the tumour cells are the infectious agent (McCallum and Jones, in press). Cancers that can be directly transmitted between hosts in wild populations are only known from two cases, Canine Transmissible Venereal Tumour (CTVT) (Das and Das 2000; Murgia et al. 2006), and DFTD (Pearse and Swift 2006; Murchison 2009). While CTVT may have had devastating population consequences when it first arose, over the 7,000-70,000 years since its inception it has evolved into a highly virulent but not lethal cancer (Murchison 2009; Belov 2010) and host recovery after infection confers immunity (Das and Das 2000; Murgia et al. 2006; Murchison 2009). DFTD is therefore the first clonally transmissible cancer in wildlife threatening its single host with extinction and the first case of a contagious cancer in which it is possible to observe evolution in progress.

The disease has proven to be consistently fatal and is characterized by fast growing tumours that usually become visible around the face, neck and oral cavity (Fig. 1.2) and often metastasize to lungs and lymph nodes (Loh et al. 2006). The latent period of DFTD is unknown and could be highly variable depending on a number of factors such as the genotypical and immunological response of the infected host, the number and location of cells transferred or overall individual fitness. To date, there are no prospects for treatment or vaccines that could be applied in wild populations in the short term (but see Woods et al. 2007).

Table 1.2 Wildlife cancers affecting species of conservation concern

Species	Conservation status (IUCN)	Tumour	Viral agent	Reference
Green turtle (*Chelonia mydas*)	Endangered	Fibropapilloma	Herpesvirus	Lu et al. 2000
Manatee (*Trichechus manatus*)	Vulnerable	Papilloma and Fibropapilloma	Papillomavirus	Bossart et al. 2002
Narwhal (*Monodon monoceros*)	Near Threatened	Papilloma and Fibropapilloma	Papillomavirus	Geraci et al. 1987
Beluga whale (*Delphinapterus leucas*)	Near Threatened	Gastric Papilloma	Papillomavirus	De Guise et al. 1994
Sperm whale (*Physeter catodon*)	Vulnerable	Genital Papilloma	Papillomavirus	Lambersten et al. 1987
Western barred bandicoot (*Perameles bougainville*)	Endangered	Papillomatosis Carcinomatosis	Papilloma and Polyoma	Woolford et al. 2008
Tasmanian devil (*Sarcophilus harrisii*)	Endangered	Devil Facial Tumour Disease	None found	Hawkins et al. 2006

The transmission dynamics and population impacts of DFTD are consistent in all populations for which medium to long-term data are available. Affected populations typically exhibit high disease prevalence in sexually mature individuals shortly after disease arrival (McCallum et al. 2009). A steady decline in adult survival rates two to three years after the epidemic outbreak results in drastic and sustained declines in population size and growth rate (Lachish et al. 2007). Age structure is subsequently affected with high mortality in adults and populations undergoing a reduction in mean age towards younger individuals (Lachish et al. 2009). Reproductive compensation occurs via an increase in precocial breeding (decline in the age of first breeding in females, Jones et al. 2008a) and female biased offspring sex ratio (Lachish et al. 2009). However, none of these compensatory responses have been sufficient to mitigate the population decline in affected populations (Jones et al. 2008a; Lachish et al. 2009). There is also compelling evidence from both epidemiological models (McCallum et al. 2009) and empirical data on biting patterns (Hamede et al. 2008) that DFTD is more

consistent with frequency dependent than density dependent transmission. This means that DFTD is capable of driving the Tasmanian devil to extinction (see de Castro and Bolker 2005).

Infectious diseases threaten many species with extinction worldwide (Pedersen et al. 2007). However, diseases that represent a serious extinction risk usually have one or more reservoir hosts, which provide a constant pool of infected individuals capable of maintaining a high force of infection even when the affected hosts are at extremely low densities or on the brink of extinction (Lopez et al. 2009). To my knowledge, DFTD is the first case of a wildlife disease threatening its single host with extinction. As there has been no indication of recovery after infection, predictions of the epidemic outcome and transmission dynamics might be dependent on factors such as genetic variability in the immune system, heterogeneities in contact rates, behavioural reduction of infection risk, or host-pathogen coevolutionary adaptations. The severe and ongoing declines that DFTD has caused in Tasmanian devil populations highlight the importance of investigating the epidemiological parameters and ecological processes involved in disease transmission. This will be critical for assessing possible disease control actions and management strategies, which are broadly applicable to other species threatened by infectious diseases.

A)

B)

Figure 1.2 Tasmanian devils showing DFTD: A) early stage of the tumour below the lower lip and B) advanced stage of tumour in lower jaw.

Photographs by Rodrigo Hamede.

Thesis aims and outline

The overall goal of this study was to develop an understanding of the transmission dynamics and ecology of DFTD in wild populations. I accomplished this through addressing four more specific aims: (1) to estimate the contact network of a wild Tasmanian devil population empirically and assess the relevance of network structure, demographic and seasonal dynamics in the epidemiology of DFTD; (2) to build a disease simulation network model capable of reproducing the observed dynamics of contact networks and to predict the epidemic behaviour and impact of DFTD compared with traditional compartmental disease models; (3) to follow and compare the progression, epidemiology and impact of DFTD in populations that differ in genetic structure; and (4) to estimate biting patterns and likelihood of acquiring DFTD in wild devil populations. In addition, I used the results of my thesis to assess possible management strategies aimed at improving the conservation prospects for Tasmanian devils.

These aims were achieved by taking a multi-disciplinary approach to both data collection and analysis. First, I used a novel technology - proximity sensing radio collars - which allowed estimation of contact heterogeneities in the wild in a continuous and consistent fashion throughout the sampling period. I used these data to construct individual and population contact matrices and to estimate important network metrics that are relevant for the transmission dynamics of infectious agents. Second, I further used these data to build disease outbreak simulation models with tuneable algorithms capable of parameterising the empirically observed contact heterogeneities and important epidemiological aspects of DFTD. Third, I used capture-mark-recapture analyses and thorough statistical treatment to determine the transmission dynamics and

impacts of DFTD in four well studied populations for which the natural progression of the disease has been rigorously followed either from prior to or from soon after the epidemic outbreak. I compared the effects and epidemiology of DFTD in populations that differ in pathogen strain and host immune genetic structure and diversity. Finally, I undertook an observational study aimed at determining the ecological and epidemiological processes that promote disease transmission and at estimating possible differences in the effects of DFTD due to biting behaviour in wild devil populations.

Chapters 2 and 3 of this thesis explore the role of social network analysis for understanding the transmission dynamics of DFTD. In Chapter 2, I examine the role of individual and population network metrics during and outside the mating season and its implications for disease transmission. Particularly, I examine important structural properties of network theory such as the degree distribution of the population and global network connectivity as well as the role that particular individuals, demographic groups or seasons play in the formation of social network and their structural properties. Chapter 3 uses the same data set to create computer generated disease simulation outbreaks and to predict the epidemic behaviour and the likelihood of host and pathogen extinction as well as the prognosis for coexistence between the devil and the tumour. The model also explores the role of different transmission rates and disease parameters such as the latent/infectious periods in the outcome of the epidemic outbreaks. The results of the network models are compared with traditional stochastic compartmental disease models and evaluate the importance of using individual contact heterogeneities and global network metrics for estimating the epidemic threshold of infectious diseases and its impact on devil populations.

In Chapter 4, I examine the infection dynamics, temporal changes in disease prevalence as well as the impact of DFTD in the population growth rate, size and age structure at a population in the current east/west disease front, “West Pencil Pine”. This site is located in the genetically distinctive provenance of northwestern Tasmania, which differs with eastern populations in their genetic diversity and structure. As this is the first population where the disease has encountered hosts with MHC genes differing from those of the tumour, I compared the epidemiology and impact of DFTD at this site with three other well studied eastern populations, where the progression and impact of DFTD had been followed early after disease arrival.

Chapter 5 explores the possibility that the significant differences in the epidemiology and impact of DFTD reported in Chapter 4 could be due to a behavioural response from the host at West Pencil Pine via a reduction in contact rates. Using a capture-mark-recapture epidemiological study I compare the biting patterns at three month intervals in all individuals between West Pencil Pine and another eastern population affected by DFTD. Furthermore, I assess whether biting intensity is a predictor for acquiring DFTD and the possible ecological or epidemiological mechanisms involved in disease transmission.

Finally, Chapter 6 synthesizes the relevance of the above four research chapters for understanding the ecology and epidemiology of wildlife diseases and its potential implications for designing management strategies and disease control actions. I discuss the implications of my results with emphasis on identifying those management actions that can mitigate the impact of DFTD and improve the conservation prospects for Tasmanian devils. I also discuss areas that deserve future research and set objectives

and research priorities for improving the management and understanding of DFTD and other diseases affecting wildlife populations.

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CHAPTER 2

Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using Social Network Analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease.

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Abstract

The structure of the contact network between individuals has a profound effect on the transmission of infectious disease. Using a novel technology - proximity sensing radio collars, we described the contact network in a population of Tasmanian devils. This largest surviving marsupial carnivore is threatened by a novel infectious cancer. All devils were connected in a single giant component, which would permit disease to spread throughout the network from any single infected individual. Unlike the contact networks for many human diseases, the degree distribution was not highly aggregated. Nevertheless, the empirically derived networks differed from random networks. Contact networks differed between the mating and non-mating seasons, with more extended male-female associations in the mating season and a greater frequency of female-female associations outside the mating season. Our results suggest that there is limited potential to control the disease by targeting highly connected age or sex classes.

Introduction

Social relationships and contact networks have profound implications for the transmission of infectious diseases (Altizer et al. 2003b). Although measuring individual contact patterns in wild animals is difficult, particularly for nocturnal forest-dwelling species, this information is critical for understanding animal sociality and disease dynamics and for building epidemiological models. The simplest models of infectious diseases assume that mixing within the host population is homogeneous. These models have proved very useful in both understanding epidemic behaviour and in guiding management interventions (Anderson and May 1991), but including more

realistic heterogeneity in contacts can have profound effects on model predictions (Keeling 2005). Modifications can be made to allow for both spatial heterogeneity and for differences in contact rates between classes of individuals. In the last decade, advances in network theory have provided powerful new tools for understanding the effect of demographic and individual heterogeneities on transmission dynamics (Lloyd-Smith et al. 2005; Bansal et al. 2007).

Determining the structure of a contact network relevant to the transmission of infectious disease is difficult even for human diseases (Bansal et al. 2007) or diseases of livestock (Vernon and Keeling 2009). For wildlife disease, these problems are magnified. Very few studies have successfully described a contact network for any wildlife disease, although Böhm et al. (2009) have recently described a direct contact network for bovine TB based on intra-and inter specific contacts between badgers (*Meles meles*) and cattle using proximity sensing radio collars. In other cases "contact" has been indirectly inferred. Porphyre et al. (2008) investigated the influence of contact rates in possums (*Trichosurus vulpecula*) on R_0 for bovine TB using a network derived from animals being caught at the same trap location within a certain time frame or animals sharing the same activity range. Cross et al. (2004) inferred contact between African buffalos (*Syncerus caffer*) using radio tracking to determine that animals were present in the same herd at the same time.

In this paper, we describe a contact network for wild Tasmanian devils derived from a novel technology, proximity sensing radio collars (see Prange et al. 2006). These are capable of logging when, and for how long, two animals have been in close proximity and therefore can determine the structure of a contact network relevant for a directly

transmitted infectious disease. Tasmanian devils, the largest surviving marsupial carnivore, are threatened with extinction by an infectious cancer, Devil Facial Tumour Disease (DFTD) (Hawkins et al. 2006; McCallum et al. 2007; McCallum 2008), thought to be spread between individuals by biting (Pearse and Swift 2006). Transmission depends on transfer of live tumour cells from an infected devil through the skin of a susceptible host. It appears that these foreign cells are not rejected by the recipient devil because of very low genetic diversity in devils (Jones et al. 2004), particularly at the Major Histocompatibility Complex (Siddle et al. 2007).

By deploying proximity loggers on the majority of individually marked adults in a population, we collected detailed information on individual and sex specific contact behaviours on a 24-hour basis. Devils are nocturnal and, whilst they are usually solitary (Jones 1998; Owen and Pemberton 2005), they interact aggressively resulting in bite injuries both around prey carcasses and during the mating season (Hamede et al. 2008). Prior to the innovation of proximity loggers, information on individual contacts was limited to behavioural observations of feeding interactions at carcasses of prey. A previous study demonstrated that injurious biting contact between devils, the type of contact that is likely to result in transmission of DFTD, occurred at a higher frequency during the mating season than at other times of the year (Hamede et al. 2008). Wild devils, however, mate in underground burrows, so information on individual and sex specific mating contacts in wild devils is sparse. Contact data collected continuously through reproductive phases may reveal temporal heterogeneities in network structure with implications for disease transmission.

There are at least two reasons why understanding the structure of the network of contacts may be of critical importance for practical management of a wildlife disease. First, it is usually the case that a small number of highly connected individuals, often described as "superspreaders", are responsible for the majority of disease transmission (Woolhouse et al. 1997; Lloyd-Smith et al. 2005). If these individuals belong to identifiable sex or age classes, then management actions such as selective culling or targeted prophylactic treatment directed at these age or sex classes may be effective in controlling transmission of the disease through a population. Second, elimination of any infectious disease from a population requires reducing the basic reproductive number (R_0) of the disease to below 1 (Anderson and May 1991; Roberts 2007). This means that estimating R_0 is important for evaluating the efficacy of potential control actions. Most estimates of R_0 are based on a mean field assumption and may be quite misleading when applied to nonrandom contact networks (e.g. Meyers 2007). For example, a high degree of heterogeneity in contacts between individuals will cause R_0 to be greater than would be predicted from mean field assumptions (May 2006; Cross et al. 2007), whereas a high proportion of triangles in the network (known as "transitivity" in the network literature) means that an infected animal is likely to be connected to other infected animals even when disease is rare, leading to R_0 being lower than would be predicted from a random network (Keeling 1999).

In this study we used social network analysis to: 1) measure association preferences and the frequency and duration of contacts between males and females during and after the mating season; 2) examine the differences in key network metrics between our observed networks and random generated networks, and 3) identify the qualitative role that individual devils play in their social network.

Methods

Proximity loggers

We used proximity data loggers (Version 2.16, Sirtrack Ltd, Havelock North, New Zealand) fitted to individual devils to record social interactions. When two loggers or more are within a set UHF radio wave detection range, the time, date, encounter length and the unique proximity logger number are recorded and stored on each data logger's memory. Data were downloaded in the field to a laptop computer once the animal was trapped. The collars also had an embedded VHF antenna, which transmitted a standard VHF signal pulse allowing for radio-location of individuals for collar retrieval. The total collar assembly weighed 120 grams.

As DFTD is a directly transmitted disease that requires physical contact for transmission, proximity loggers were programmed to detect and interrogate each other at a separation distance of about 30cm or less (see Supplementary Information Appendix S2.1), a range at which devils can physically touch and bite each other. Detectors were set up to have a separation time of 10 seconds, meaning that a single continuous encounter was recorded until the receiving collar(s) failed to detect the transmitted signal for a period longer than 10 seconds.

Before deploying the proximity collars in our wild population we tested the detection distances in laboratory trials and in captive devils held at the Tasmanian devil Conservation Park (see Supplementary Information Appendix S1 for details on proximity logger's performance and data handling).

Study site and trapping protocol

We undertook this study within Narawntapu National Park, at that time a 25km^2 disease free devil population in northern Tasmania (41º 07′ 58′′, 146º 39′ 24′′ E). Devils were trapped according to protocols detailed in Hawkins et al. (2006). Proximity loggers were fitted on devils during January 2006 with data collected for analysis from February to June 2006. This period was chosen so that differences in contact behaviour between mating (February-March) and non-mating seasons could be assessed. We collared all sexually matured devils (≥ 2 years old) trapped prior to the mating season in January 2006; a total of 46 adults (23 males; 23 females). Collared devils were re-trapped during March, April and May to test collar performance and to determine the reproductive status of females. Proximity collars were retrieved for data analysis during July 2006.

Determination of mating and non mating season

We determined the mating season interval in this study by visually scoring the appearance of the female's pouch (see Hesterman et al. 2008a) and by backdating, based on the size and stage of development of the pouch young (see Guiler 1970b) to estimate birth dates. The pouch exhibits a marked morphological development during the mating season including an increase in size, intense coloration and secretory activity associated with different stages of the oestrous cycle and pregnancy, enabling reproductive status to be ascertained from a pouch inspection in the field (Hesterman et al. 2008a). Mating occurs over a period of approximately three days, and with delayed ovulation and a gestation period of 14-22 days (Hesterman et al. 2008b) , the total time from mating to birth is 28-31 days (Guiler 1970b).

Network construction and statistical analyses

We constructed social networks and estimated association preferences based on contact matrices directly derived from proximity loggers. Because logged encounters were not symmetrical and because our short detection distance likely meant that animal movement or alignment might break single actual contacts into separate segments, we scored the length of contact as the union of a contact recorded between two collars. This meant that a single contact was recorded as commencing when either of two collars logged the other and was terminated when neither of the two collars had logged the other for a period of at least 10 seconds). To explore and compare different qualitative and quantitative aspects of our proximity logger data, we constructed two sets of networks; one based on the total number of encounters and another based on the sum of encounter length of each pairwise interaction. Network diagrams were created using the program NetDraw (Bogartti 2002). Randomizations and statistical analyses were undertaken using code written in R (version 2.5.1) specifically for this purpose.

Randomisations

To generate null models of random association we employed the 'trial-swap' matrix randomisation algorithm developed by Miklos and Podiani (2004). The resulting set of permuted matrices does not have the potential bias of the widely-used 'swap' algorithm (Manly 1995), although the 'trial swap' algorithm is more computationally intense.

Using this procedure, Monte-Carlo simulations with different numbers of steps were performed repeatedly in order to determine the effects upon p-values, calculated for test statistics discussed below. For all devil contact networks, simulations containing 80,000 iterations of the observed devil presence-absence matrix ensured that the associated p-values had standard errors of between 1-3%.

Interaction patterns

To test for non-random, sex-based interactions at the population level the 'mixing matrix' methods of Newman (2003) were used. In this approach an assortativity coefficient, *r*, measuring the preferences of animals to mix with others in their own demographic, is computed. When this is combined with a standard error estimate σ^2_r, one can calculate confidence intervals for the observed pattern of preferential mixing, as follows.

Let *n* be the total number of edges in the observed contact network, and let m_{ij} be the fraction of edges within the network which connect individuals of type *i* to those of type *j*. The assortativity coefficient is defined as

$$r = \frac{\sum_i \{m_{ii} - (\sum_j m_{ij})(\sum_k m_{ki})\}}{1 - \sum_i (\sum_j m_{ij})(\sum_k m_{ki})\}} \quad (1)$$

where *i* and *j* correspond to either male or female classes. The quantity *r* is conceptually similar to Pearsons's correlation coefficient: it takes values $-1<r<1$, with positive and negative values respectively signifying that within-class contacts are preferred or avoided. The magnitude of *r* determines the strength of the association, with values close to zero suggesting random contact patterns.

The standard error is estimated by calculating r_k, the value of *r* for the contact network which has the k^{th} edge deleted, for each value $k=1, \ldots, n$:

$$\sigma^2{}_r = \sum_{i=1}^{n} (r_i - r)^2 \quad (2)$$

At the level of individual contacts it is common to measure associativity using a pairwise Association Index (*AI*) (Ginsberg and Young 1992). Letting c_{ij} be the interaction strength between individuals *i* and *j*, the simple ratio *AI* is defined as

$$AI_{ij} = \frac{c_{ij}}{c_{ij} + \sum_{k} c_{ik} + \sum_{\ell} c_{\ell j}} \quad (3)$$

Using the Monte Carlo process described above we generated networks consisting of identical numbers of males and females keeping the number of between-gender and within gender contacts constant .The proportion of such randomly-generated networks in which a given contact has a smaller *AI* value than the empirically observed one can hence be used to define a significance threshold. In this way it is possible to identify "preferred" contacts (Sundaresan et al. 2007), that is those having an *AI* value outside the 95% confidence intervals of the corresponding range in the null model. Such contacts may be interpreted as pairs of animals which are likely to avoid (low *AI* values) or prefer (high *AI* values) contact with one another.

Role of individuals and gender within the contact network

To test for gender-based differences in behaviour we computed a number of network metrics quantifying different aspects of an individual's importance to overall network structure. Firstly, we calculated 'node degree', which calculates the number of connections that an actor has in the network (i.e. the number of contacts a devil makes or alternatively, the total time with which it is in contact with other devils). Then we estimated the 'betweenness' of individuals, which counts the number of shortest contact paths (geodesics) between other pairs of animals, which have it as an intermediary contact. Finally, we tested 'information centrality' which is essentially a generalisation of betweenness, counting all paths running through an individual, and therefore accounts for how close a gatekeeper animal is, on average, to other animals in the network.

Within-population, sex-related differences for node-based metrics were analysed using a permutation test. For our network metrics, we compute the likelihood that the difference in mean values for each sex, could have arisen through random association.

Results

Data coverage

We recovered complete data sets from 27 proximity loggers, (15 females and 12 males); three loggers had irreparable data corruption and for two loggers the memory was full before the end of the mating season. Four collared road-killed devils were found in the vicinity of the Park during the study period with incomplete or destroyed data. One devil was trapped without its proximity sensing radiocollar, while a further two loggers were found in the landscape with incomplete data sets. Seven loggers were not recovered. We only analysed proximity loggers with complete data sets.

Determination of mating season and non mating season

During both January trapping trips (January 6th-15th and January 22nd -31st) no female (n=23) exhibited morphological signs of pouch development associated with the oestrus cycle (see Hesterman et al. 2008a). At the end of March (March 27th – April 2nd), 14 of the 16 females from which we recovered complete proximity logger data exhibited pouch characteristics of having been in oestrus. Of the remaining two females, one did not apparently come into oestrous and did not breed (a five year old female) and one was detected with a delayed or second oestrous (morphological signs of pouch development associated with oestrus detected on May 10th).

The first pouch young from a female from which we recovered data, was recorded on April 25th (small young; <15 mm. crown –rump; no pelage; undeveloped mouth and eyes) and were estimated to be a maximum of 20 days of age (see Guiler 1970b). From an approximate parturition date of April 5th, we deducted the estimated length from mating to parturition (31 days, Guiler 1970b) to obtain the start of the mating season as February 5th. On June 6th the last female from which we recovered data was captured with pouch young also estimated to be a maximum of 20 days. We calculated parturition, pregnancy and oestrous periods as described above to obtain the end of the mating season as April 17th. These mating season estimates cover the broadest spectrum in which females could have been receptive to males. The timing of this mating season in wild devils concurs with other published literature for this species (Guiler 1970b; Hughes 1982; Hesterman et al. 2008a).

Interaction patterns

In both mating and non-mating seasons, the devil contact network had a single giant component: all the collared devils were connected to all other individuals, either directly or via intermediate animals (Figs. 2.1 and 2.2). However, some of these connections either occurred only a few times or were of very short duration (frequency distributions of contact lengths are shown in Supporting Information Fig. S2.1). Figure 2.3 shows degree distributions of contact networks inside and outside the mating season for networks based on all contacts (top row) and for networks filtered by removing either low numbers of contacts or contacts of short total duration. If occasional or short total duration contacts were removed, some individuals were isolated from all other individuals. In the unfiltered networks, the variance of the degree distribution was greater than 95% confidence intervals from random networks with the same number of

nodes and edges (Table 2.1), indicating aggregation. However, coefficients of variation were relatively small. Compared to random networks, the observed networks had higher levels of transitivity (the fraction of connected triples of nodes which form triangles, Keeling 1999), higher levels of betweenness and lower mean degree. When networks were filtered, there was less evidence of aggregation and the observed networks were indistinguishable from random networks (Fig. 2.3, Table S2.1).

'Preferred' interactions within seasonal networks identified by the AI index are shown on Figs. 2.1 and 2.2. During the mating season, all preferred associations but one (a female-female association) corresponded to inter-sexual interactions whether the network was based on the number of encounters (Fig. 2.1a) or the sum of encounter length (Fig 2.2a). However, outside the mating season, preferred associations for the number of encounters network were dominated by intra-female interactions, followed by inter-sexual and lastly by intra-male preferences (Fig. 2.1b), whereas for the sum of encounter length network intra-female and inter-sex associations were equally preferred and intra-male associations were comparatively rare (Fig. 2.2b).

One three-year-old male (individual 20) had a high value of betweenness, both inside and outside the mating season, indicating a central role in the contact network. However, the identity of other individuals with high levels of betweenness differed between the mating and non-mating seasons, with no obvious difference between the sexes or age classes.

A) **B)**

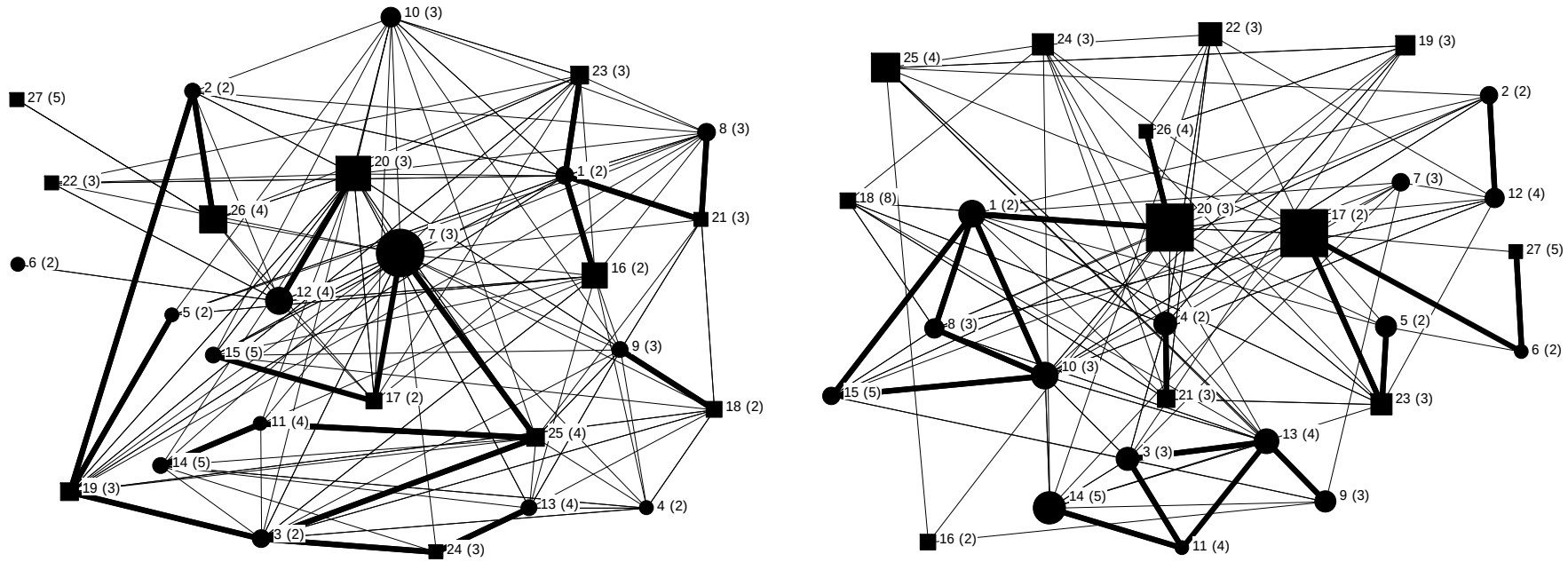
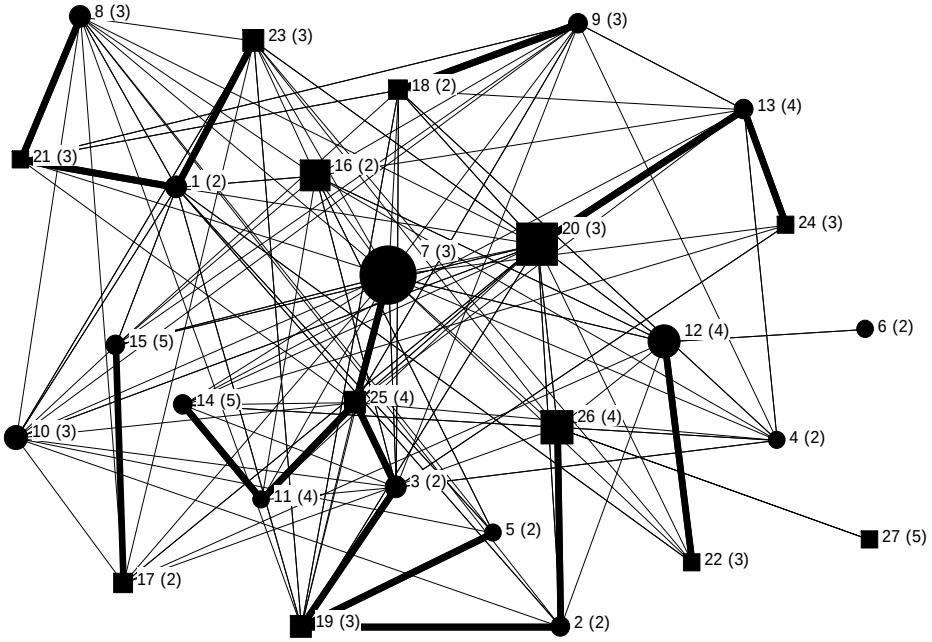


Figure 2.1

Observed contact networks of female (circle) and male (squares) Tasmanian devils during the mating season (A) and after the mating season (B), based on number of encounters. Size of symbols is determined by node betweenness, numbers beside symbols are individual's identification tags and numbers inside brackets represent individual's age in years (maximum longevity in wild devils is between five and six years). Thin lines represent individuals having a contact at least once while thick black lines represent 'preferred' (AI) interactions. Networks were drawn using the 'spring embedding' algorithm to determine the layout of nodes and edges, with minor manual adjustments.

A)



B)

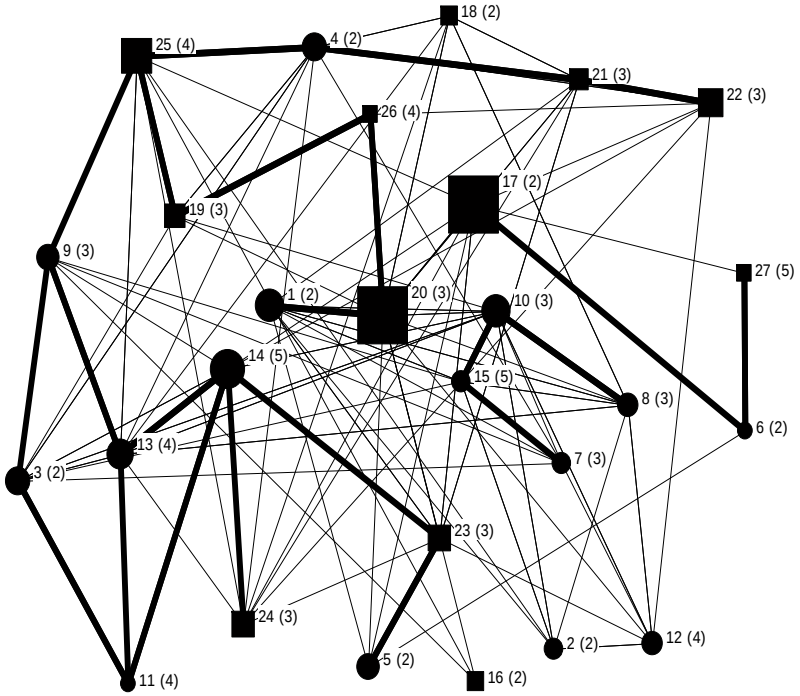


Figure 2.2

Observed contact networks for female (circle) and male (squares) Tasmanian devils during the mating season (A) and after the mating season (B), based on sum of encounter length. Symbols, edge thicknesses and node labels are as in figure 1.

Table 2.1 Comparison of whole-network metrics for the mating and non-mating season networks, compared to the average of 10000 random networks containing the same number of animals and contacts.

Network metric	Mating season	95% CI	Non-mating season	95% CI
Mean degree	12.7	12.7[a]	10.4	10.4[a]
Degree variance	34.9	(10.2,29.3)	20.3	(8.6,25.6)
Mean betweenness	26.6	(22.5,25.0)	25.1	(24.7,30.7)
Transitivity	0.47	(0.18,0.30)	0.40	(0.13,0.26)

[a] The randomization algorithm preserves the numbers of nodes and edges, and hence mean degree.

Sex-preferred associations and assortative coefficient values for the two different networks (sum of encounter length and number of encounters) are summarised in Table 2.2. In both networks, there were statistically significant differences between association preferences during mating and non mating seasons, as evidenced by the non-overlapping 95% confidence intervals. In both networks, inter-sex contacts during mating season were preferred ($r<0$). Outside the mating season, inter-sex encounters were preferred in the sum of encounter length network, but assortativity was random in the count of encounters network. In terms of intra-sex preferences, intra-female associations were more likely outside of the mating season, whereas intra-male associations tended to be less common.

Table 2.2 Measures of association preferences (r) and percentage of sex-based associations during both seasons. Note that both estimates of contact (count of encounters and sum of encounter length) have strong mixing preference for inter-sexual contact and intra-sexual avoidance during mating season.

Season and quantity measured	Assortativity coefficient (r)	95% CI	Male-Male	Male-Female	Female-Male	Female-Female
Mating sum of encounter length	-0.98	(-0.98,-0.98)	<0.01	0.48	0.51	0.01
Mating count of encounters	-0.94	(-0.94,-0.93)	0.01	0.46	0.51	0.02
Non-mating sum of encounter length	-0.36	(-0.37,-0.35)	0.11	0.33	0.34	0.22
Non-mating count of encounters	0.02	(0.00, 0.04)	0.15	0.22	0.23	0.40

Network metrics and the role of sex and individuals in the contact network

Mean values of individual-based network metrics were calculated for both sexes and subjected to a permutation test based upon the 'trial swap' algorithm. For none of the node metrics used could a significant difference in behaviour between the sexes be identified on the basis of a P=0.05 significance threshold (Table 2.3). This may indeed be due to the absence of sex-related differences, although the presence of one well-connected individual of each sex would tend to skew mean quantities and mask any effects. Due to the small sample size, these significance tests have relatively weak statistical power.

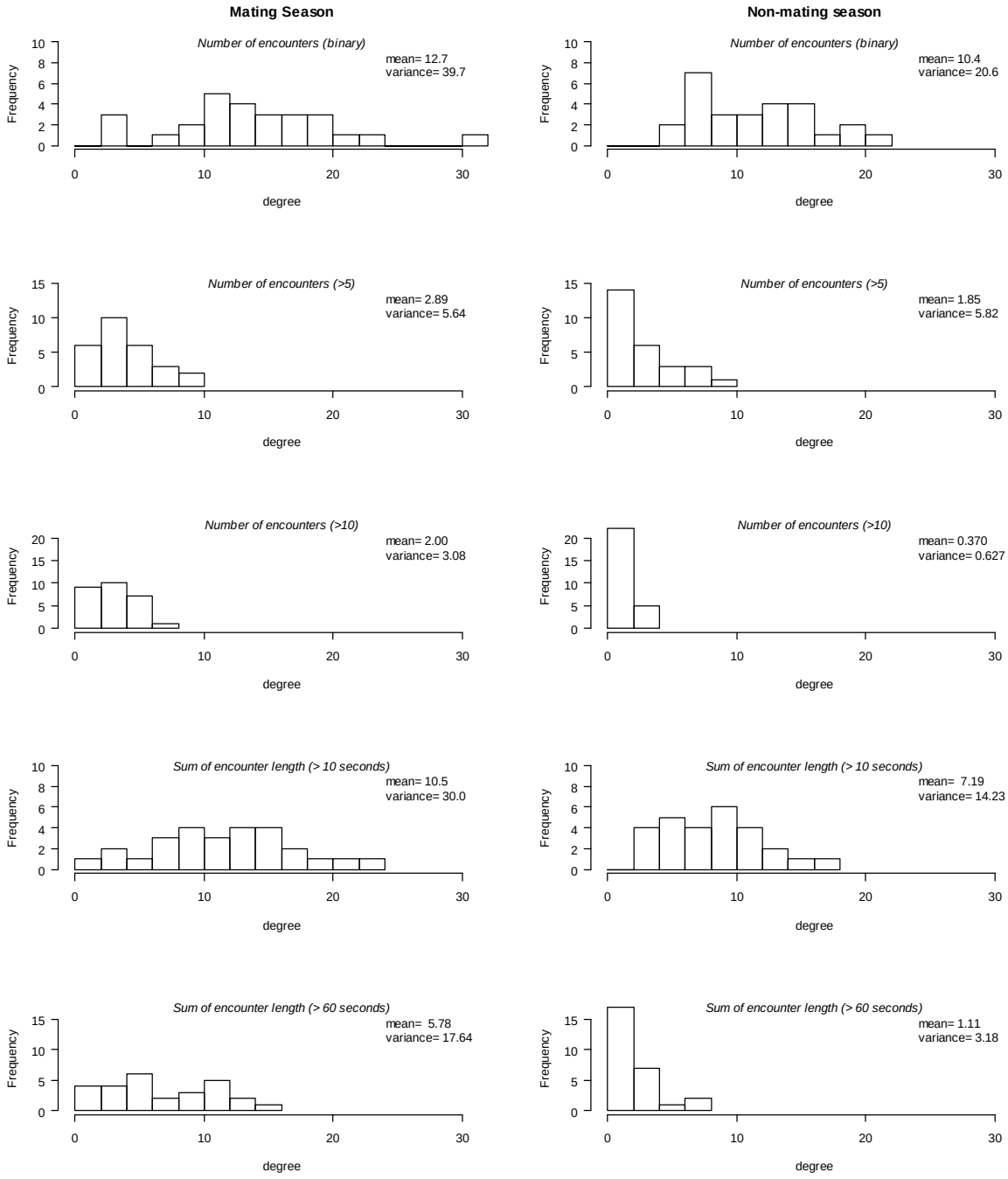


Figure 2.3

Degree distribution of nodes during mating and non mating seasons with different threshold values for "contact". Thresholds based on number of encounters are shown in the three top rows and thresholds based on the sum of encounter length are shown in the two bottom rows. The mean and variance of each degree distribution are also shown on the figure.

Table 2.3 Descriptive statistics of observed differences between males and females in the mean values of individual-based network metrics. Confidence intervals and p values describe distribution of randomly permuted networks.

Network metric	X_0 (observed)	95% CI	$P(X_0>X_{random})$
Mating season			
Degree	-0.73	(-3.43, 3.46)	0.32
Betweenness	-16.7	(-38.9, 36.4)	0.26
Information Centrality	-0.04	(-0.23, 0.23)	0.69
Non-mating season			
Degree	-1.3	(-3.8, 4.0)	0.29
Betweenness	-2.6	(-23.5, 24.4)	0.41
Information Centrality	0.10	(-0.30, 0.36)	0.61

Discussion

Most empirical data on networks relevant to disease transmission has been collected for human diseases and has involved asking individuals to recall their social contacts or using diaries to record social contacts as they occur (Keeling and Eames 2005; Mikolajczyk and Kretzschmar 2008). The limited existing analyses of contact networks relevant to the transmission of wildlife disease have largely been based on inferring contact through animals occupying the same general area within some particular time period (Cross et al. 2004; Porphyre et al. 2008; Perkins et al. 2009). The proximity sensing radio collars we used in this study provide data similar to that derived from contact diaries in human disease by directly recording actual contacts of relevance to transmission of DFTD. To our knowledge, this is the second study (see Böhm et al. 2009) in using these contact sensing collars to derive a contact network for a wild population, although Ji et al. (2005) used them to produce a contact network for captive

brushtail possums and Prange et al. (2006) used them in wild raccoons to assess proximity collar's performance in the field.

The contact networks revealed by proximity collars have several features that increase understanding of potential DFTD transmission. Despite Tasmanian devils usually being described as "solitary" (Russell 1984; Owen and Pemberton 2005), all individuals in our study population, both in the mating season and outside, were connected to a single network. DFTD in any one individual could therefore spread across the entire group. The mating system of Tasmanian devils is poorly understood, though described by Russell (1984) as promiscuous. Our contact matrix derived from the mating season (Fig. 2.2a, Table 2.2) reveals preferred associations between male-female pairs, which might represent male guarding of females. These extended contacts may facilitate transmission. Male – female preferred associations are much less evident outside the mating season (Fig. 2.2b). Outside the mating season, there was still evidence that the length of contacts was greater between rather than within the sexes (Table 2.2). Somewhat surprisingly, male-male interactions were relatively uncommon, both within and outside the mating season, suggesting that males tended to avoid each other. Outside the mating season, contacts between females were relatively common (Table 2.2), with most "preferred" interactions being between females (Figs. 2.2 and 2.3). In contrast to these sex dependent contact patterns, there is no evidence that the prevalence of DFTD differs between males and females at any of the six study sites for which long-term data are available (McCallum et al. 2009); nor is there evidence that survival of animals in infected populations differs between the sexes (Lachish et al. 2007); or that the frequency of bite injuries differs between the sexes (Hamede et al. 2008). However,

there is no simple predicted relationship between rates of contact between and within sexes and infectious disease prevalence (May et al. 1998).

There were substantial and significant differences in the network structure between the mating and the non-mating seasons (Table 2.2, Fig. 2.2). In addition, there is evidence that injury rates from penetrating bites amongst adults are higher within the mating season than at other times (Hamede et al. 2008). These seasonal patterns in contacts and injuries are not reflected in seasonality in DFTD prevalence in adult devils (McCallum et al. 2009). However, this discrepancy does not necessarily mean that there is no seasonality in transmission as the incubation period of DFTD is unknown. If the incubation period has a distributed delay, then it may obscure seasonal variation in the transmission rate.

The degree distribution was not highly aggregated, either within or outside the mating season (Fig. 2.3). This is a surprising result, as degree distributions of contacts in many other species are highly aggregated (e.g. Perkins et al. 2009). A possible explanation is that Tasmanian devils do not defend exclusive territories (Guiler 1970a) and, while solitary, aggregate and interact around prey carcasses when feeding. Given that our collared animals occupied overlapping home ranges, the individuals around a carcass at any given time may represent an essentially random sample of the collared animals. Nevertheless, the results in Table 2.1 show that the contact network, both inside and outside the mating season, differs from random networks generated from the same number of nodes and edges. Using all contacts, the variance of the degree distribution in the observed networks was significantly greater than in random networks (Table 2.1), although this was not the case when networks were filtered to remove short or

occasional interactions (Table S2.1). Observed networks also had significantly higher levels of transitivity ($p<0.002$) than random networks during both seasons. Such network clustering has been argued (Keeling 2005) to help offset waves of infection, by ensuring the pathogen is confined to small, locally well-connected patches.

The basic reproductive number R_0 is strongly influenced by heterogeneity in the degree distribution and can be written as

$$R_0 = \rho_0 \left[1 + (CV)^2 \right], \quad (4)$$

where ρ_0 is a constant and CV is the coefficient of variation of the degree distribution network (May 2006).

The relatively small coefficient of variation in our contact network, coupled with the presence of clustering, suggests that R_0 is likely to adequately be predicted by the mean contact frequency alone.

One individual (male 20, 3 years old) is the most strongly connected individual (both in terms of degree and betweenness) within and outside the mating season. Betweenness measures the extent to which all connections in the network run through a particular individual, which therefore would be expected to be important in disease transmission. Individuals of high-betweenness have been identified as important in determining the extent of an outbreak, both from contact-tracing studies (Klovdahl et al. 2001) or simulations (e.e Ueno and Masuda 2008). There is also empirical evidence that individuals with high betweenness may be particularly important in disease transmission (Corner et al. 2003).

In principle, control actions targeted at highly connected individuals should be more effective at disease control than untargeted control. However, with the exception of this one individual, the identities of the other strongly connected individuals differed between the mating and non mating seasons. Further, mean betweenness in our networks, both within and outside the mating season, did not differ from that of random networks with the same number of edges and nodes. Scope for directing management at highly connected individuals is therefore limited as no particular sex or age class was obviously more highly connected than others (see Table 2.3 for a comparison of connectedness between sexes).

A limitation of almost all empirically derived networks is that not all of the population has contacts recorded. Although we successfully collared all adult Tasmanian devils caught in the study area, data were not recorded from all of them, with 27 of the 46 collars deployed returning complete data. Although Tasmanian devils are highly trappable using the methods employed in the study (see Lachish et al. 2007), not all adult devils in the area at the time collars were deployed would have been captured. Further, the devils in our study could have interacted with individuals on the periphery of the study area. The network inevitably is thus a subset of the full contact network. Nevertheless, we can think of no reasons why edge effects, failure to catch all resident adults in the initial field trip, collar loss or collar failure would discriminate between individuals of differing contact patterns.

Transmission of infectious disease is a three stage process. First, a susceptible and an infected individual need to make sufficiently close contact for potential transmission of infectious agents. Given that devils frequently bite each other when in close contact

(Hamede et al. 2008), our proximity sensing radio collars have measured the relative risk of this stage of the transmission process with greater precision than have previous studies in other species that have relied on individuals occupying the same general area within some common timeframe (e.g. Cross et al. 2004; Porphyre et al. 2008). The second stage of transmission is that the contact needs actually to result in the transfer of infectious agents from infected to the susceptible host. Our study has produced no information on this stage. The biology of DFTD requires that live tumour cells should be transferred from the infected to a susceptible host, most likely by a penetrating bite. How long a contact is required for there to be a high probability of a penetrating bite and what proportion of contacts result in such a bite is unknown. The third stage of transmission is that once the infectious agent is transferred, it needs to develop into a new infection. Again, we have no information on the stage. Only by following a novel infection or infectious strain as it passes through a known network can information be gained on the second and third stages. This has rarely been done in any wild population, although Corner et al. (2003) did so for bovine tuberculosis in captive brushtail possums and Otterstatter and Thomson (2007) followed the transmission of an intestinal pathogen through bumblebee hives. Ethical considerations have precluded doing a similar study on DFTD in Tasmanian devils.

In conclusion, our study has produced a social network for a wild species subject to an emerging disease, based on actual contacts rather than simple co-occurrence of individuals in the same general area within some specified time period. The fact that all individuals are connected to a single giant component shows that the disease is capable of spreading to each individual in the population once one individual is infected. Although the devil network has a small proportion of individuals with high betweenness

values, no particular age class or sex appears to be especially highly connected, which limits the possibility of disease suppression by targeting for removal particular age or sex classes. Intensive trapping and removal of all infected individuals captured from a population has not yet proved to be capable of suppressing disease on an isolated peninsula (Jones et al. 2007). The disease, however, remains at low prevalence in individuals younger than two years of age (McCallum et al. 2009). Females usually do not reproduce at less than two years of age, although there are indications of increased first-year female breeding in infected populations (Jones et al. 2008). Removal of all two year old and older individuals may therefore be required to suppress disease, although whether this would permit the persistence of Tasmanian devil populations is unclear. Disease suppression would be enhanced by development of a test capable of detecting infected devils before they become infectious. In the absence of such a test, management needs to concentrate on isolating currently unaffected Tasmanian devils from disease and in exploring the possibility that there may be genotypes sufficiently different from the tumour cell line that they are resistant.

Supplementary Information

Appendix S2.1 *Proximity Logger Information*

Proximity loggers (Sirtrack Ltd, Version 2.16) are radio collars that can communicate with each other over a short range radio data link. They can be set up to detect each other at pre-set threshold distances by adjusting the power setting of a UHF coefficient range. This coefficient ranges from 31 to 62 with the lowest value representing the highest distance at which loggers will record encounters (Sirtrack Limited 2006). Each logger broadcasts and receives unique identification codes over a UHF channel at 1.5 second intervals, allowing the loggers to simultaneously send and receive other UHF signals (Prange et al. 2006). A contact or encounter begins when two or more loggers detect the signal of another within the pre set detection distance and ends when the receiving logger fails to detect the signal for a pre set period of time ("separation time"). The date and time of detections of other proximity loggers are recorded in UTC format and are corrected to local time after the data are retrieved. Loggers can detect and log a maximum of eight other collars simultaneously. When this maximum is reached the subsequent logger is ignored. This ceiling is unlikely to be exceeded in our study as devils are solitary carnivores that rarely form large aggregations. A previous study in seven different populations (including our collared population) never recorded more than four individual devils feeding at the same prey carcass (Hamede et al. 2008).

Before deploying proximity collars in the wild population, we undertook laboratory and field trials to test their performance. All collars were manually adjusted to a UHF power setting of 45, to provide a detection threshold distance of approximately 30 cm. In the laboratory trials, collars were tested by moving pairs of loggers towards each other to

different minimum approach distances. All pairwise combinations of all collars (N=54) were randomly for testing of detection distance. The same test protocol was repeated with simultaneous testing of randomly selected sets of 3 and 4 collars. Field trials were undertaken using captive devils at the Tasmanian devil Conservation Park. Proximity loggers were fitted to two, three and four devils simultaneously and collared devils were video-filmed while feeding on a prey species carcass. A stopwatch was used in both laboratory and field trials to record encounter duration and large black and white measuring stick was set in the background to assist with identifying distances in the field.

Forty-seven proximity loggers successfully detected each other in the laboratory trials at 30cm or less and start failing to detect each other at 40cm or more. Of the remaining seven loggers, four were not consistently receiving UHF signals at the set detection range and three were not sending a UHF signal. These loggers were excluded from the field study. In field trials collars successfully detected each other at an estimated distance of 30 – 40cm and start failing at 50 – 60cm or more.

Proximity loggers have been observed occasionally to record *phantom contacts*; one second duration encounters incorrectly decoded as a result of weak signal strengths when the collars are at or just outside the continuous detection range (Prange et al. 2006, Sirtrack Limited 2006). In our laboratory and field trials, the proximity loggers recorded one second encounters at distances ranging from 30 - 110cm, and from 30 - 90cm, respectively. Consequently, all contacts of one second duration (32% of all encounters) were removed from our final data set.

UHF radio waves can be affected by factors such as the height of the transmitter above the ground, and absorption and/or reflection by surrounding objects. The data, therefore, cannot be interpreted as representing exact spatial precision or to match identical contact durations between pairs of collars/animals (Prange et al. 2006; Sirtrack Limited 2006, Böhm et al. 2009). While in theory, two or more collars that are placed within the detection range should log the same encounter length, in laboratory and field trials we recorded discrepancies in contact length between pairs of collars of between 1 and 12 seconds over a range of up to 60 seconds duration contacts recorded using a stop watch. To account for the discrepancy in contact length logged on a pair of collars, we used the union, or non-overlapping part of the two time intervals. In addition, events which occur within the programmed collar separation time (10 seconds) were combined into a single encounter bout.

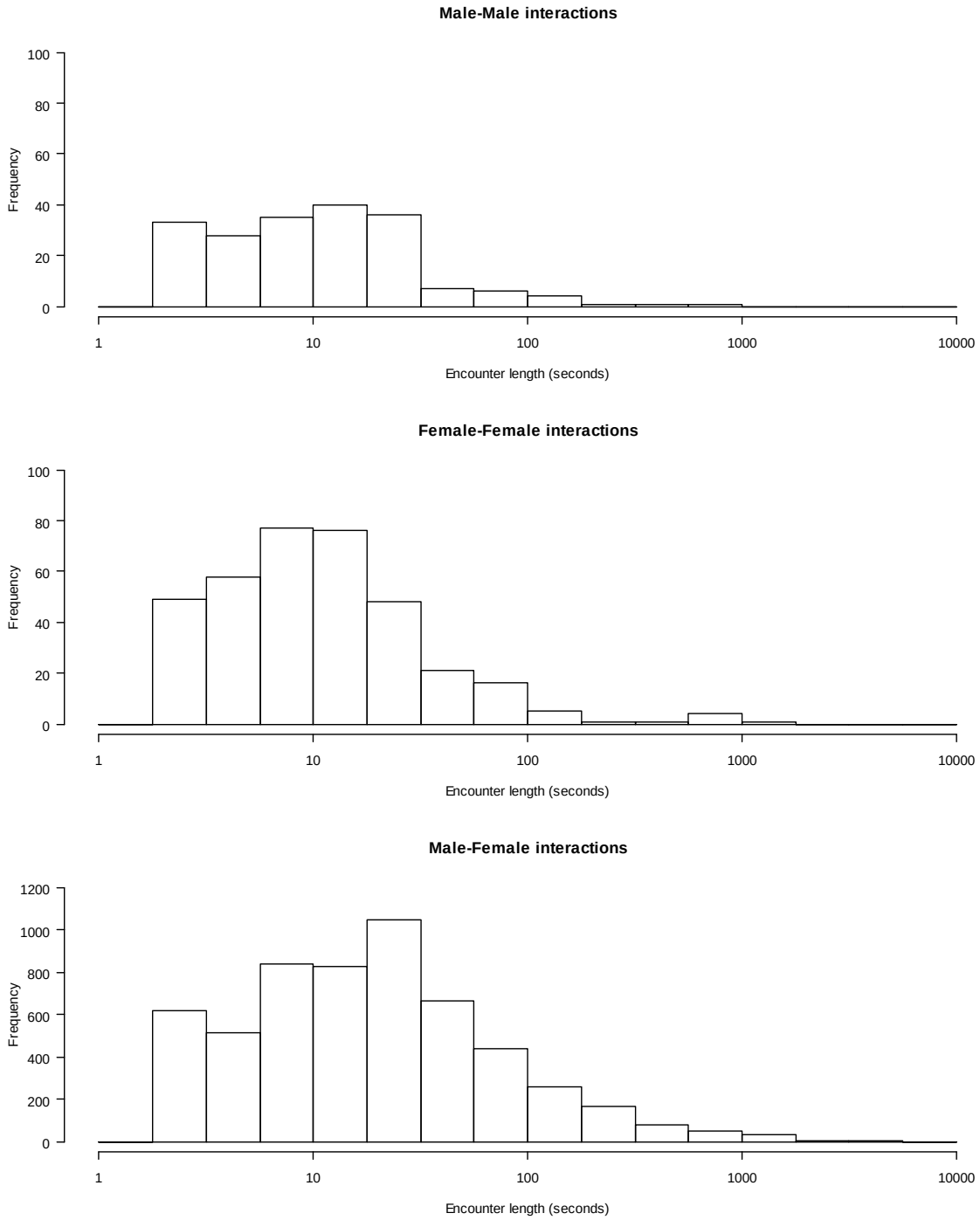


Figure S2.1

Frequency distributions of pairwise contact lengths comparing male-male, female-female and male-female encounters.

Table S2.1 Comparison of whole-network metrics for the filtered, mating and non-mating season networks appearing in the lower eight panels of Figure 3. Observed values are compared to the average of 2000 randomly-drawn networks containing the same number of animals and contacts.

Number of encounters >5	Mating season	95% CI	Non-mating season	95% CI
Mean degree	2.9	2.9[a]	1.8	1.8[a]
Degree variance	5.2	(2.9,8.7)	4.9	(8.3,24.9)
Mean betweenness	10.4	(5.6,53.4)	1.07	(24.8,30.7)
Transitivity	0.10	(0.00,0.24)	0.30	(0.00,0.26)
Number of encounters >10				
Mean degree	2.0	2.0[a]	0.37	0.37[a]
Degree variance	3.2	(2.3,6.0)	0.55	(0.72,1.33)
Mean betweenness	2.7	(1.0,16.6)	0.00	(0.00,0.30)
Transitivity	0.00	(0.00,0.27)	-[b]	0.00
Sum of encounter length > 10 seconds				
Mean degree	10.5	10.5[a]	7.2	7.2[a]
Degree variance	20.4	(8.5,26.0)	14.0	(6.8,18.5)
Mean betweenness	29.7	(24.4,30.1)	33.9	(29.9,45.0)
Transitivity	0.42	(0.13,0.26)	0.37	(0.05,0.22)
Sum of encounter length > 60 seconds				
Mean degree	5.8	5.8[a]	1.1	1.1[a]
Degree variance	9.5	(5.6,15.8)	1.8	(1.3,3.5)
Mean betweenness	38.1	(30.1,58.9)	0.92	(0.07,2.30)
Transitivity	0.30	(0.03,0.21)	0.19	(0.00,0.29)

[a] The randomization algorithm preserves the numbers of nodes and edges, and hence mean degree.

[b] No triangles in observed network.

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CHAPTER 3

Simulating devil facial tumour disease outbreaks in weighted networks: the role of contact heterogeneities in epidemic behaviour.

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Abstract

1. Understanding the nature and characteristics of contact heterogeneities is crucial for predicting the epidemic behaviour of infectious diseases. Nonetheless, few studies include contact heterogeneities when modelling disease outbreaks in wildlife, which differ in their population impact from human diseases.

2. We use empirical estimates of contact heterogeneities and network metrics to simulate outbreaks of Devil Facial Tumour Disease (DFTD), an extinction-threatening infectious cancer. We incorporate tuneable algorithms, with a range of transmission rates and latent periods of DFTD, to grow devil population networks capable of reproducing observed aspects of devil ecology, demographic and seasonal based mixing preferences. The outputs of the network model are compared with a stochastic mean field model, in which every individual is equally likely to pass or acquire infection through time.

3. Our network model predicts a lower epidemic threshold for DFTD compared with the stochastic mean field model. While host extinction probabilities are similar in both models, the network model predicts faster devil extinction and higher DFTD extinction probabilities, particularly for intermediate levels of transmissibility.

4. While the time taken to devil extinction increases with the longer estimate of latent period, probabilities of both, disease and devil extinction, are greater with the shorter latent period. Host-pathogen coexistence is strictly subject to the longest plausible estimate of latent period and low transmissibility.

5. *Synthesis and applications.* In the particular case of DFTD, incorporating observed host network structure has only a modest effect on the outcome of the host pathogen interaction. Estimating network structure and modelling its implications for disease dynamics should, nevertheless, be an important step in developing management

strategies for emerging infectious diseases in wildlife. Obtaining information on the transmission dynamics and epidemiology of DFTD across different genotypic populations and tumour strains should become a priority to further assess the effect of potential management strategies in the conservation of the species.

Introduction

Most compartmental models of disease (eg. Anderson & May 1979) make a mean field assumption, which is that disease dynamics can adequately be modelled by assuming that individuals make contact with each other at random. Estimates of the basic reproductive number R_0 derived from such models form the basis of most management strategies, because elimination of a disease requires driving R_0 to below one. However, it has become increasingly clear that the structure of many contact networks for both human and wildlife diseases indicates that mean field assumptions can lead to unreliable estimates of R_0 (Meyers *et al.* 2005; Keeling 2005; Porphyre *et al.* 2008). In addition, incorporating network analysis in epidemiological studies can help to identify how pathogens can avoid threshold density effects.

Modelling disease spread through contact networks is a recent approach to understanding disease spread and transmission, which can explicitly account for non-random mixing patterns of hosts, and is thus becoming a powerful predictive epidemiological tool (Gomes-Gardeñes *et al.* 2008; Mosson *et al.* 2008; Eames, Read & Edmunds 2009). Contact networks can be used not only to investigate epidemiological parameters of an infectious disease but also to identify key individuals or core groups ("superspreaders") in epidemic outbreaks, to predict their impact on disease dynamics and to design targeted control strategies (Christley *et al.* 2005; Bohm, Hutchins &

White 2009). All models must make assumptions about contact patterns between individuals, either by incorporating certain heterogeneities in contact or by assuming random mixing. The use of social network analysis has been a major step towards assessing the validity of the assumption that the mean contact rate between individuals is adequate to describe transmission dynamics. The application of network theory in human epidemiology has proved to be extremely useful for estimating individual heterogeneities in contact patterns and examining their implications for disease transmission dynamics for both sexually transmitted (Liljeros *et al.* 2001; Gomes-Gardeñes *et al.* 2008) and non sexually transmitted diseases (Anderson *et al.* 2004; Lloyd-Smith *et al.* 2005).

The epidemiology of wildlife diseases is qualitatively different from that of most human diseases because in wildlife diseases, particularly highly pathogenic ones such as devil facial tumour disease (DFTD), the population size is strongly affected by the disease. This may have a major influence on the structure of the network itself. Difficulties in collecting data on contact networks in free ranging animals have hampered application of network approaches to understanding wildlife diseases. However, recent advances in radio telemetry technologies combined with the emerging interest in network theory have allowed the collection of more accurate information on animal contact networks in wildlife disease studies (Bohm, Hutchins & White 2009; Hamede *et al.* 2009).

Social networks often differ from many other types of technological or biological networks in two important structural properties: high levels of clustering (termed ‘transitivity’, which is a network metric that estimates the density of triangles in a network) and a positive correlation between the degrees (or the number of connections)

of neighbouring nodes (Newman & Park 2003). This means that individuals are more likely to be connected in the network if 1) they share a mutual neighbour or 2) if they have a similar number of contacts. Such network properties will evidently play important roles in determining the likelihood of an epidemic outbreak and in mediating infection dynamics.

A common assumption when modelling epidemics through networks is that all interactions (whether direct contact, close proximity of individuals, or shared/overlapping use of resources) have the same probability of causing infection. This is clearly not the case for transmission of many parasites and pathogens (Perkins, Ferrari & Hudson 2008; Drewe 2010). For instance, for diseases that require direct contact for transmission (such as DFTD) detailed information on the social setting of interactions is needed (Hamede *et al.* 2009). This is in contrast to diseases that can be spread through aerosol spray or fomites, such as bovine Tb, in which it may be reasonable to assume that animals sharing the same home range or trapping grid over a certain period are at greater risk of passing infection (Porphyre *et al.* 2008). These differences in the quality and frequency of interactions within a contact network can be modelled by giving each interaction a 'weight' which may relate to the frequency of the contacts, the total time spent with other individuals, the proximity or the level of intimacy of such encounters (Read, Eames & Edmunds 2008).

The Tasmanian devil (*Sarcophilus harrisii*), the largest living carnivorous marsupial, is currently threatened with extinction by DFTD, a fatal infectious cancer (McCallum & Jones 2008; McCallum *et al.* 2009). DFTD is transmitted by direct inoculation of tumour cells between infected and uninfected hosts through biting (Pearse & Swift

2006), a process facilitated by very low host genetic diversity at the Major Histocompatibility Complex (MHC) genes associated with tumour recognition (Siddle *et al.* 2007). Decreased survival and population growth rates in affected populations predict that local extinctions are possible within 10 years of disease arrival (McCallum *et al.* 2007). We recently derived an empirical contact network for wild Tasmanian devils, obtained from a novel technology (proximity sensing radio collars) (Hamede *et al.* 2009), in which we characterized seasonal contact network dynamics in a distinct population. Our key question in this paper is to determine how the structure of this observed contact network might affect the dynamics and epidemic behaviour of this disease in the wild and hence potential management strategies.

In this study, we simulate the epidemic spread of DFTD into naïve devil populations using weighted networks which incorporate gender mixing properties and seasonal shifts in network metrics associated with mating behaviour (Hamede *et al.* 2009). We use tuneable algorithms for growing virtual networks capable of recreating our observed devil contact patterns into epidemic simulations. We run these epidemic simulations by including population and disease parameters into a susceptible-exposed-infected (SEI) network model to investigate seasonal, sex and population-based network metrics on spread of DFTD under different transmission rates and latent periods of the disease. Finally we compare our results and estimations of R_0 with those of a stochastic mean field model, in which every individual is equally likely to pass or acquire infection through time.

Materials and methods

Model structure

The devil population is described by a basic age-structured stochastic population dynamic model, which determines the number of nodes (devils) at any point in time (see Supplementary Information). Empirically determined seasonal and sex-mixing preferences (Hamede *et al.* 2009) were used as input for an algorithm to create networks with realistic metrics (mean degree and transitivity: see table 1). To incorporate the effects of seasonal and sex-based contact patterns within this population, we use a formula for probabilistically joining a pair of nodes. Consider a square of side length $\sqrt{K}$ (hence yielding an area of K units) wrapped into a torus to avoid boundary effects, where K is the carrying capacity of the landscape. After assigning random uniform coordinates to each node within this area, the probability p_{12} that two nodes are joined by an edge depends on i) *dist*, their separation in the landscape and ii) the (empirically-estimated) rate of association between their sex classes:

$$p_{12} = \begin{cases} 1 & \pi_{12} \geq 1 \\ \pi_{12} & \text{otherwise} \end{cases}$$
$$\text{where } \pi_{12} = e_{AB} \exp(-(dist)^2 / 2V) \qquad (1)$$

where A and B denote the sexes of the two animals. Here e_{AB} represents the affinity for an animal of sex A for an animal of sex B. The *Gaussian width* parameter V can be thought of as representing either animal ranging or network clustering. Essentially for $V \sim 0.003K$, long-range contacts within a set of K animals (in this paper, the population at carrying capacity) occur at a rate expected for a random collection of nodes (Badham, Abbass & Stocker 2008). For V values below this value (short-range) clustering is preferred (Fig. S3.1a) and the network giant component (the connected sub-network

containing the majority of the nodes in the entire network) is likely to be small. Conversely as V increases beyond this threshold, any node is likely to be joined to any other node and the model approaches mean field (hereafter 'MF') behaviour (Fig. S3.1b). This is borne out in Figure S3.2, which shows the dependence of giant component and mean degree upon V. In this study we set V=0.003K, consistent with the assumption that neither 'long range' nor 'short range' contacts are preferred. Once a choice for V has been made, the affinities e_{AB} are scaled so as to generate networks with metric values (mean degree, degree correlation) comparable to those observed empirically (Hamede *et al.* 2009).

As described in the supplementary material, births, deaths and disease transmission are propagated in these simulated contact networks using the 'next-reaction' formalism of Gillespie (1977). 'Reactions' correspond to transitions between susceptible, exposed and infectious classes. The basic approach was modified to account for i) spatial structure in terms of network connectivity and ii) the need to include age-dependence in natural devil mortality. Parameter definitions and initial values used for the simulations are presented in table 3.1, whereas figure 3.1 shows the basic model structure.

Devil population models

The stochastic algorithm we used to build dynamic contact networks is able to capture several aspects of devil ecology and DFTD transmission. Based on the length of the mating season and observed changes in sex-based mixing preferences (Hamede *et al.* 2009), we set the 're-wiring' period (the interval after which the network edges were regenerated from the algorithm described earlier) at three months (see Supplementary Information). This means that for every (annual) recruitment event, there are four seasonal re-wiring events. Then, the 'full network' model consists of three consecutive

seasons where non-mating season mixing parameters determine patterns of contact followed by a fourth season where the annual recruits appear and mating season mixing quantities are used to grow the network. In addition, we examined the effects of three other network re-wiring schemes: 'season only' (mating versus non-mating mixing preferences), 'sex only' (male versus female mixing preferences), and non-preferential mixing ('rewiring effect only'). Given that we have empirically estimated heterogeneities in contact patterns in sexually mature devils only (Hamede *et al.* 2009), and that prevalence in animals less than two years of age is very low (McCallum *et al.* 2009), recruitment into the network model occurs only once devils reach sexual maturity (at 2 years of age).

Finally, for comparison to the established approach in network modelling, we consider the MF model population. That is, network structure is ignored and all susceptible animals are equally likely to contract DFTD from an infected devil, regardless of location, season or sex.

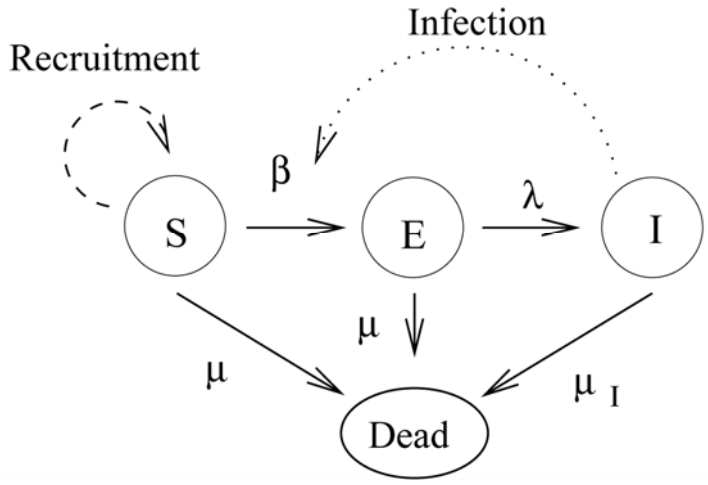


Figure 3.1 Compartmental model with the transitions of susceptible, exposed and infected classes. Ageing of each class is given by the Gompertz parameter *G* described in table 1. The parameter *ß* (transmission rate) is replaced for *T* (transmission probability) in the network model.

Estimates of Latent Period

The latent period of DFTD is unknown but is likely to be variable depending on a number of factors such as the genotype and immunological response of the infected host, the number and location of cells transferred or overall individual fitness. Our estimates of latent period are inferred form the best information available based on field (R. Hamede, unpublished data) and experimental observations (Kreiss *et al.* 2010; S. Pyecroft, unpublished data). There is one anecdotal case of a wild Tasmanian devil brought into captivity which developed DFTD ten months after its removal from the wild (Tasmanian Department of Primary Industries, Parks, Water and Environment, unpublished manuscript). Experimental inoculation of tumour cells in captivity have shown a much faster but still highly variable latent period, although the dosage used in these trials was considerably higher than what could be expected in a 'natural' transmission scenario (Kreiss *et al.* 2010). Field observations of individuals that have been monitored through time with DFTD in several populations suggest that tumours can increase ten-fold in size in as little as three months and can cause death in six – twelve months (R. Hamede, unpublished data). In our simulation models we use four different estimates of latent period which range from three to twelve months.

Estimating R_0

We used several methods of estimating R_0 as a function of different contact patterns. For basic reference we use a stochastic SEI compartmental model assuming MF behaviour. By analogy with a deterministic SEI model with frequency dependent transmission, this basic estimate is

$$R_0 = \frac{\beta}{\mu_I} \frac{\lambda}{\lambda + \mu} \qquad (2)$$

where β represents the infection rate (transition from susceptible to exposed classes), λ is the (exponentially distributed) rate of transition through the exposed class to the infected class, μ is disease independent mortality and μ_I is the mortality rate of infected animals (for a full definition of μ and μ_I see *Host population dynamics* in Appendix S1). We estimate this MF R_0 for our randomly generated networks by making the following substitution:

$$\beta \to 4T\langle d \rangle$$

where the angled brackets represent the mean (infected) node degree per unit time and T is the probability per unit of time that a transmission event occurs, given two nodes are connected (here the factor of 4 arises in converting from contacts per three-month season to an annual transmission rate).

Anderson *et al.* (1986) suggest that heterogeneity in contacts can be accounted for by adjusting the mean field approximation of R_0 as follows:

$$R_0^A = (1 + \frac{\sigma_d^2}{\langle d \rangle^2}) R_0 \tag{3}$$

whereas Newman (2002) suggests the following

$$R_0^N = \frac{\langle d(d-1) \rangle}{\langle d \rangle^2} R_0 \tag{4}$$

In both equations (3) and (4), the angled brackets denote globally-averaged quantities and σ_d is the standard deviation of the degree distribution. The superscripts A and N differentiate the arguments used by and Anderson *et al.* (1986) and Newman (2002) respectively.

For each simulation we record the lifespan of each animal and, when infection occurs, the time it spends in the exposed and infected classes. By extracting transition rates (μ, λ, μ_I) from each simulation we are able to incorporate the effects of intra-specific regulation and ageing into our estimates of R_0. At the beginning of each simulation we assumed that 97% of the population was susceptible and 3% was infectious. Based on field observations (R. Hamede, unpublished data) the value of induced mortality μ_I was kept fixed (6 months).

Quantity	Symbol	Value
Number of adult devils	N	-
Number of reproducing females	n_F	-
Carrying capacity	K	100 animals
Annual recruitment rate	R	1–2 female^{-1} $year^{-1}$
Natural mortality rate	m	0.07–0.12 season^{-1}
Gompertz parameter	G	1–2
Gaussian variance parameter	V	0.003K
Mating gender-mixing ratios	$e_{MM} : e_{MF} : e_{FF}$	1: 3.5: 1.1
Non-mating mixing ratios	$e_{MM} : e_{MF} : e_{FF}$	1.5: 3: 2.2
Re-wiring interval	δt_{seas}	1 season
Recruitment interval	δt_{rec}	4 seasons
Transmission rate (MF)	β	0-4 seasons^{-1}
Transmission probability	T	0-1
Latency rate	λ	0.25–1 season^{-1}
Induced mortality	m_I	0.25 season^{-1}
Mean mating season degree		14
Mean non-mating season degree		10
Mean mating season transitivity		0.47
Mean non –mating season transitivity		0.39

Table 3.1 Parameters and symbols used in network and mean field models.

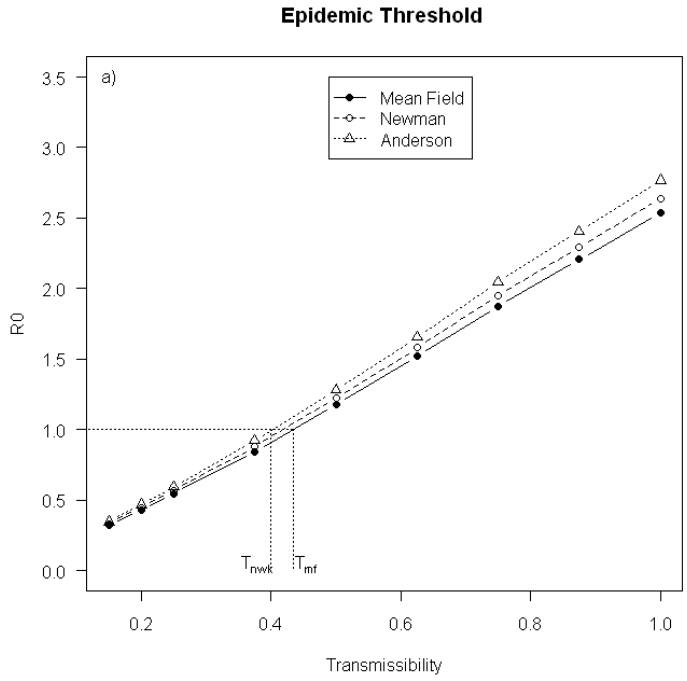
Results

Three different outcomes after 50 years are possible: devil extinction, devil coexistence with disease and devil persistence with disease extinction. For each set of values for transmission probability (T) and latent period of DFTD (λ), 500 simulations with identical conditions were used.

Estimation of R_0

Figure 3.2a shows the differences in the value of R_0 (and hence the epidemic threshold) for network and MF models for a latent period of 6 months. Essentially, incorporating empirically-derived devil contact patterns increases the R_0 of DFTD by between 5 and 15%, depending on whether the estimates of Newman (2002) or Anderson *et al.* (1986) are used. Accordingly the epidemic threshold value of transmissibility is ~10% lower than the MF estimate, $T_{mf} \sim 0.44$.

Figure 3.2b shows analogous plots, but for a latent period of 9 months. From equations (3) and (4) it follows that the network R_0 values are again 5-15% larger than the MF prediction. However increasing the latent period has marginally raised the (MF) epidemic threshold, $T_{mf} \sim 0.47$.



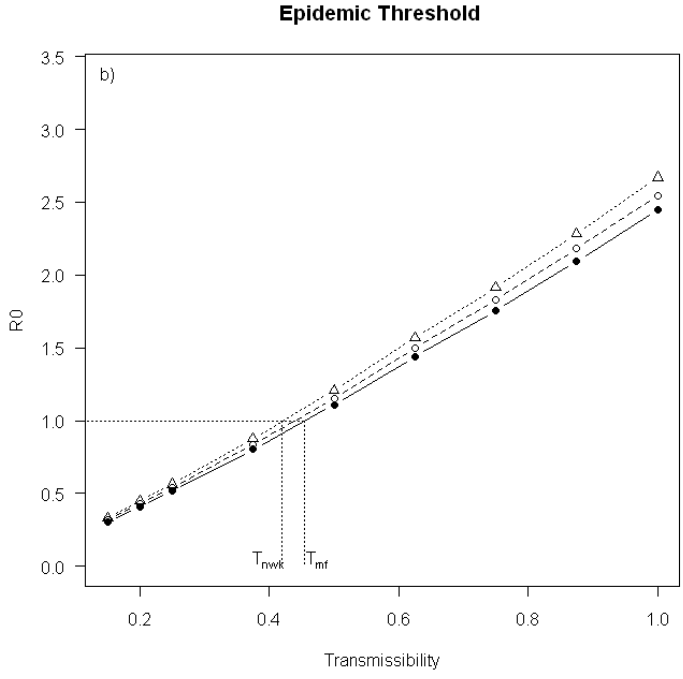


Figure 3.2 Estimates of R_0 (median) and epidemic threshold (R_0 =1) in the three methods using 2 different latent periods, A) = 6 months, B) = 9 months.

Effect of contact patterns in disease simulation models

To demonstrate the effect of contact patterns on disease transmission and epidemic outcome we ran simulations comparing MF models to the network model. Additionally, we considered scenarios where neither preferences ('rewiring' effect only), one of (either 'season' or 'sex' only) or both ('full network') sex and seasonal dependent association behaviours were present. Predictions of the probability of devil extinction were higher at intermediate values of transmission rate in the MF model, but similar between both models at the lower and upper values of transmissibility (Fig 3.3a). The predicted time to host extinction is faster in the network model than in the MF model but this difference between models converges as transmission rate approache one (Fig. 3.3b). The lower values of transmissibility, at which these large differences between models in devil extinction processes occur produce estimates of R_0 (see figure 3.2a) that are inconsistent with the observed estimates from field data (McCallum *et al.* 2009).

Unsurprisingly, host-pathogen coexistence was only possible at low to intermediate values of transmissibility, as higher values of transmissibility inevitably led to the extinction of either host or pathogen (Figs 3.3a and 3.4b). There was a large difference between the MF and network predictions for the probability of host-pathogen coexistence (Figure 3.4a). This result shows that the constraints imposed on connectivity between hosts in the network models directly increase the likelihood of DFTD extinction at the expense of devil-DFTD coexistence (Fig 3.4b) while at the same time accelerating the timescale of host extinction processes (Fig 3.3b). For both probability of host-pathogen coexistence and probability of pathogen extinction, the difference between the models converges at higher transmissibility values (Fig. 3.4a and 3.4b).

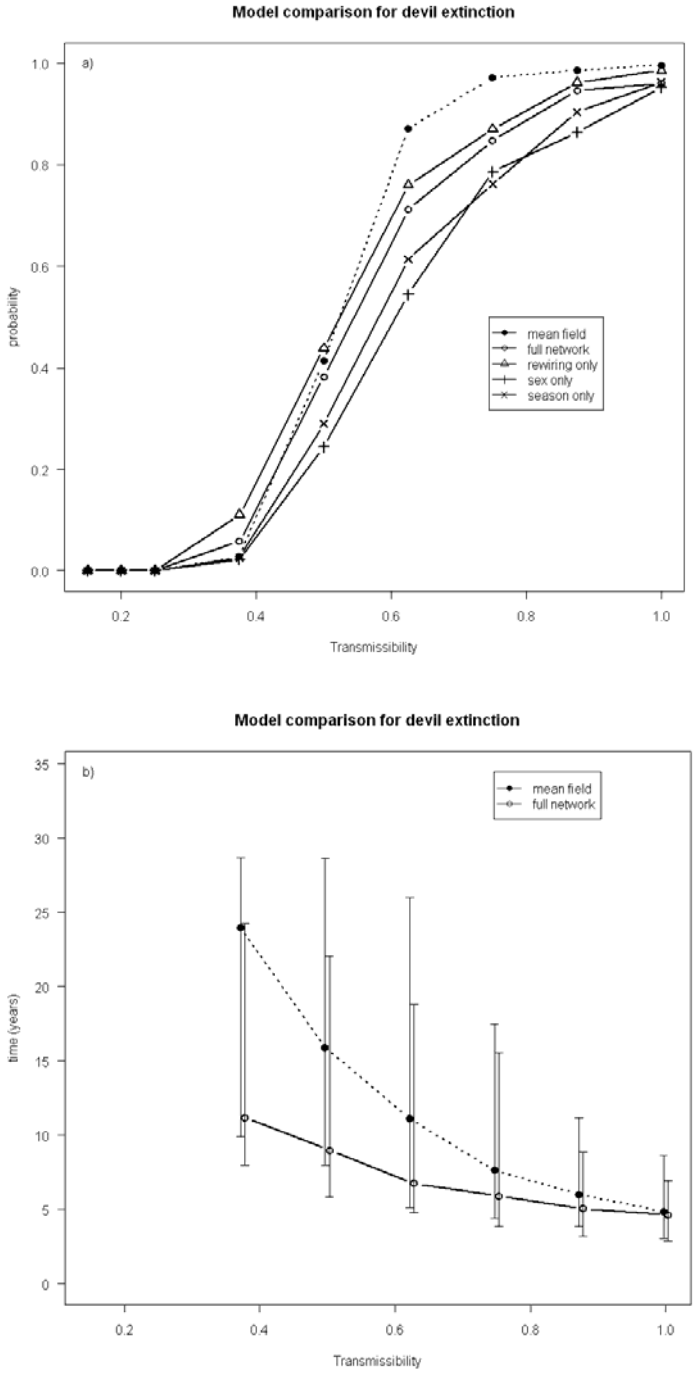


Figure 3.3 Effect of network association preferences versus mean-field behaviour for a) devil extinction probability and b) median projected time for devil extinction, (λ = 12 months).

Note: time of extinction was obtained only from cases where devil extinction occurred.

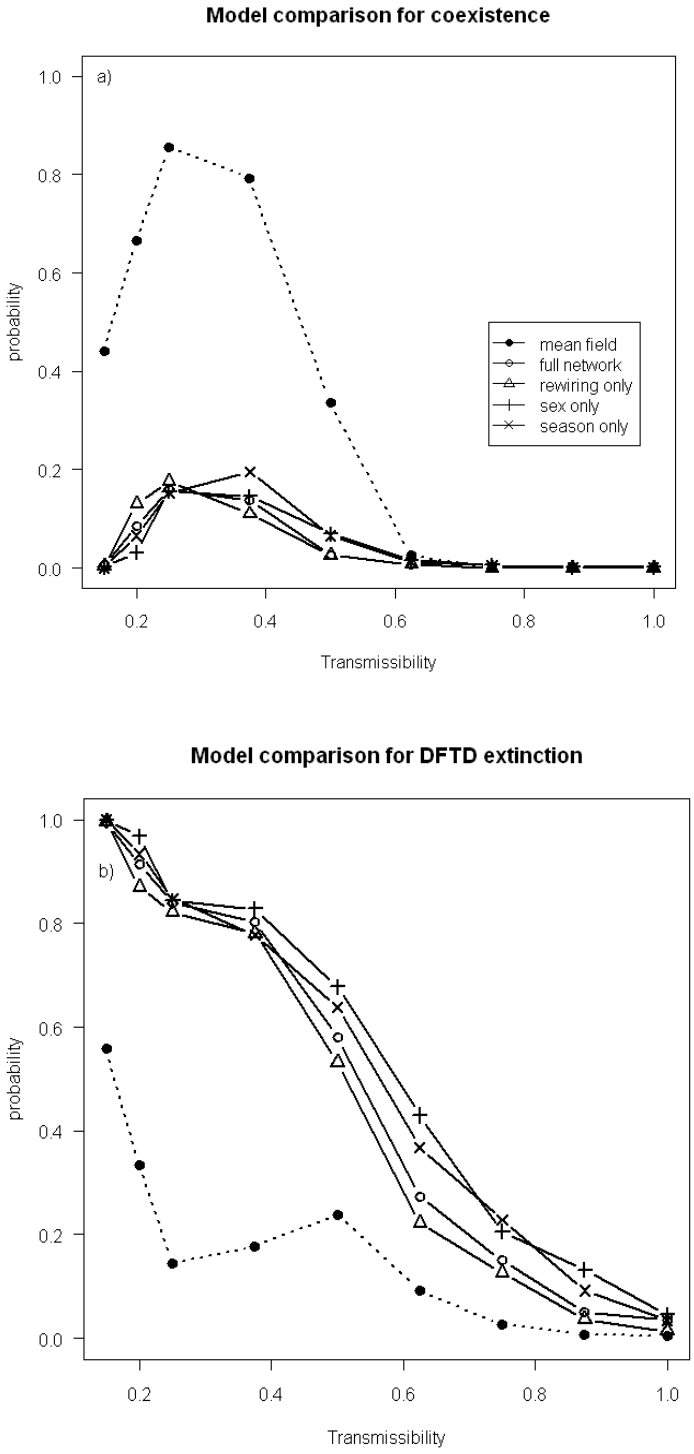


Figure 3.4 Effect of network association preferences versus mean-field behaviour for a) devil-DFTD coexistence after 50 years, b) DFTD extinction probability, (λ = 12 months).

Note: coexistence probabilities were obtained only from cases in which both devil and DFTD survived after 50 years. DFTD extinction probabilities are conditional to devil survival.

Effect of latent period

We ran simulations of our network model to estimate the effect of latent period on devil and DFTD extinction and coexistence probabilities (Figs. 3.5a - 3.5c) as well as predictions of time taken to drive either devil or DFTD to extinction (Figs. 3.6a and 3.6b) at different values of transmissibility. As was expected, a shorter latent period for DFTD increased the probability of host extinction, particularly at intermediate values of transmissibility (Fig. 3.5a). The time taken to devil extinction also increases with the longer estimate of latent period (Fig. 3.6a). Probabilities of disease extinction are greater with the shorter latent period, increasing up to ~15% at intermediate rates of transmissibility (Fig 3.5b). A similar pattern can be seen in Fig. 3.6b where the time to disease extinction increases at the intermediate levels of transmissibility, while the shorter latent period favours a faster rate of pathogen extinction.

Interestingly, host-pathogen coexistence was not possible with a short latent period regardless of the transmission rate (Fig 3.5c). With a long latent period coexistence is possible, reaching with a maximum probability of ~25% at intermediate transmissibility (Fig 3.5c).

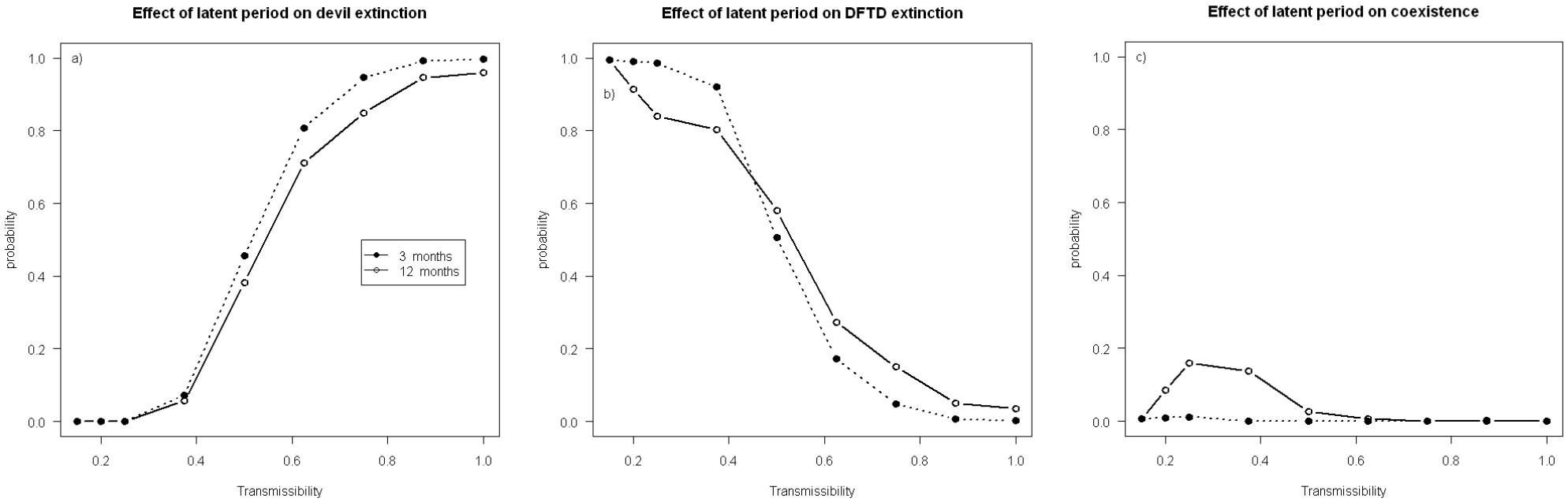


Figure 3.5 Effect of latent period on (a) devil and (b) DFTD extinction probabilities and coexistence (after 50 years) in the full network model.

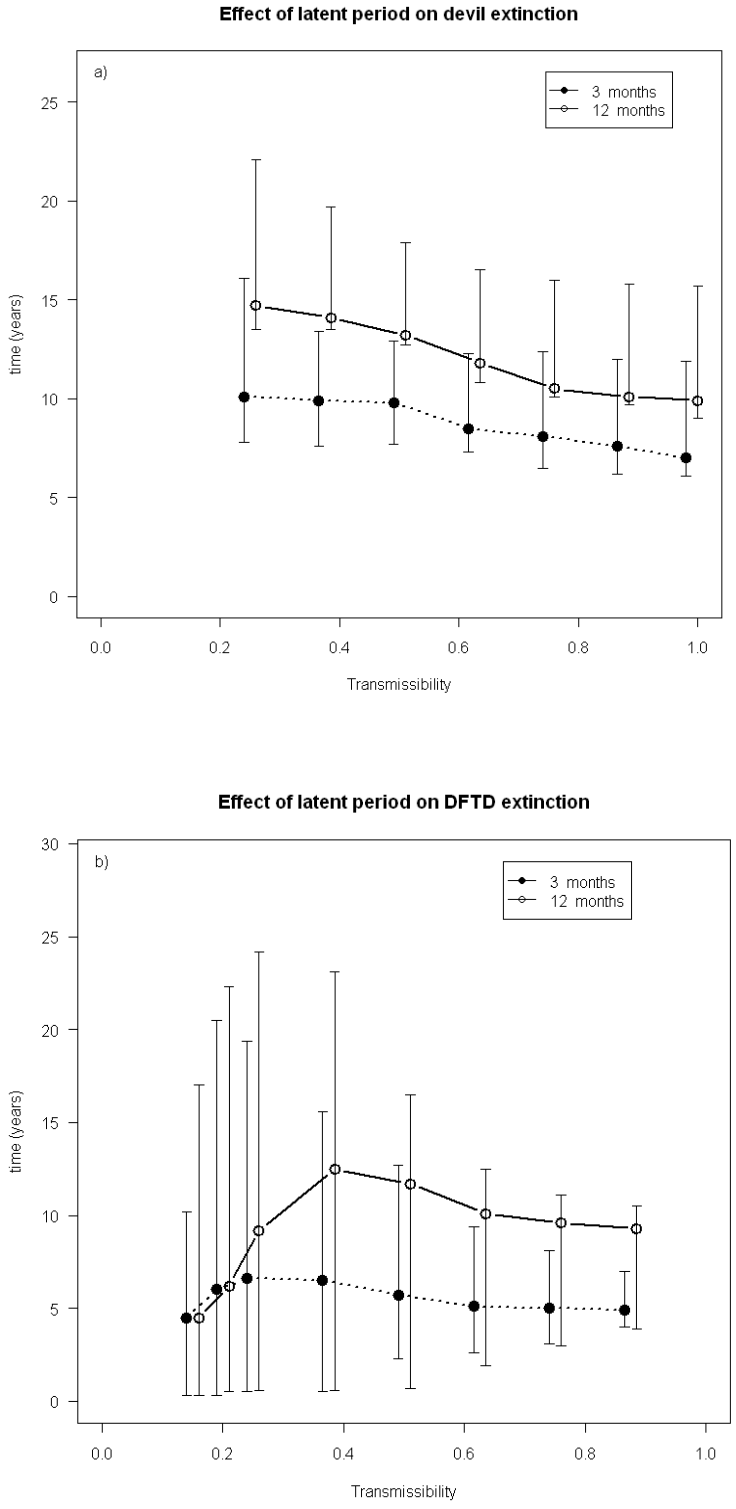


Figure 3.6 Effect of latent period on (a) devil and (b) DFTD extinction time in the full network model.

Discussion

A limited but increasing number of studies have demonstrated the profound effect that the structure of a contact network can have on the dynamics of infectious diseases (Keeling 2005; Christley *et al.* 2005; Read, Eames & Edmunds 2008). Our network modelling approach allows us to make several inferences regarding the role of heterogeneities in contact patterns on the transmission dynamics of DFTD. The most immediate conclusion we can draw from our simulation outbreaks is that our network model, that incorporates known heterogeneities in contact patterns with reproductive season and sex variation, predicts a lower transmissibility threshold for an epidemic to occur compared to the MF model (Fig. 3.2). Individuals with anomalously high number of contacts (i.e. scale-free networks) are capable of decreasing the epidemic threshold of a disease (Pastor-Satorras & Vespignani 2001) compared to randomly mixed populations. The devil social networks used in this study are designed to contain properties of scale-free networks during the mating season (eg. superspreaders), in accordance with our earlier analysis of empirical data on devil interactions (Hamede *et al.* 2009).

The values for R_0 in the network model at the intermediate estimates of transmissibility range between $0.9 < R_0 < 2.0$. However, McCallum *et al.* (2009) derived estimates of R_0 from increase in DFTD prevalence in 2-3 year old devils in two different populations. These estimates ranged between $1.6 < R_0 < 3.1$ using a six months latent period estimate and $2.1 < R_0 < 5.4$ using a nine months latent period. Extrapolating those estimates into our network simulation model with identical latent periods suggests transmissibility values for DFTD approaching to one. More accurate estimates of R_0 will depend on

better definition of the incubation and latent periods, which at present is hindered by the lack of a diagnostic test of DFTD in the absence of clinical signs.

The latent period of DFTD influences the probability of extinction of both host and tumour and the potential for coexistence between them. Devil extinction probabilities differ by only 10% depending on shorter (three months) or longer (twelve months) estimates of latent period at the intermediate values of transmissibility. However, a shorter latent period in the network model substantially hastens the process of devil extinction. Similarly, the shortest latent period increases the probability of DFTD extinction by a 15%, most likely as a result of the pathogen dying out within a local patch before it can reach the whole network. Conversely, a longer latent period permits 'exposed' hosts spending more time in such condition, allowing the pathogen to reach and spread across the entire network.

Incorporating real-world contact rates amongst devils into our network model also resulted in higher predicted probability of pathogen extinction, as a direct result of the greater seasonal and sex-based constraints on connectivity between hosts within the population. Conversely, under the MF model the chance of pathogen extinction is considerably less likely than in the network model, even when values for transmissibility approach zero. This suggests that homogeneous mixing models might tend to overestimate potential contagious contacts.

Our network models do not take into account contact patterns of juvenile devils (one year old), which are only recruited in the model when they become sexually mature in the subsequent year. However, juvenile devils usually do not engage in mating, except

in populations where older sexually mature devils have succumbed to DFTD, which results in precocial breeding and early sexual maturity (Jones *et al.* 2008). In addition, prevalence of DFTD in juveniles has been shown to be very low unless populations have been practically reduced to one year old devils only (McCallum *et al.* 2009). Further research into the role of juvenile devils in the dynamics of DFTD, once the older age classes have disappeared due to DFTD mortality, is needed to understand their role in the epidemiology of DFTD.

Network structure has implications for the likelihood of disease induced devil extinction. Extinction is slightly more likely (~10%) at intermediate values of transmissibility in the MF compared to the network model, possibly due to the higher connectivity in the MF model. Host extinction probability does not differ between the MF and the network models if values for the transmissibility are close to zero or one. This suggests that for diseases with either very high or very low transmissibility, contact heterogeneities seem to have little effect on the likelihood of disease-induced host extinction. However, transmission rates per contact are likely to be intermediate in many wildlife diseases. In the case of DFTD it is likely that only a small proportion of contacts between infected and susceptible animals will result in transmission because direct inoculation of live tumour cells (and their subsequent establishment) is necessary to acquire infection. Biting is the most likely route for transmission. Whilst biting is very common in Tasmanian devils (Hamede, McCallum & Jones 2008), not all bites result in penetration of the dermal layer (Pemberton & Renouf 1993) and have the potential to result in disease transmission. Likewise, not all contacts recorded by proximity sensing radio collars will have resulted in a bite, but conversely, some very short contacts that may have resulted in bites may not have been long enough to meet

the 10 seconds threshold we used to define a contact in our empirical devil network (Hamede *et al.* 2009). Given that our contact rate estimates were obtained form a disease-free population, further studies are needed to clarify if DFTD is capable of changing contact patterns at individual or population level.

Rewiring the network creates further opportunities for disease transmission by providing new chances of connecting individuals that were previously not connected, increasing the likelihood of devil extinction. Previous studies have demonstrated that rewiring nodes in dynamic networks have direct consequences for the spread and persistence of infectious diseases (Gross, Dommar & Blasius 2006; Volz & Meyers 2007). Rewiring the entire network was performed at discrete intervals for computational tractability but is clearly only an approximation to the actual continual changes in network structure that would occur in a real population as seasons change, animals die and new individuals are recruited. In this study, we found re-wiring to have a greater impact on simulation outcomes than the specific forms of mixing preferences (sex and/or season). Although differences in individual mixing preferences within our network model are small at most values of *T*, they reach a 15-20% difference in devil extinction probabilities when only gender or seasonal mixing preferences are compared with the effect of rewiring.

There are substantial differences between network and MF models in the probability of coexistence of host and the pathogen (defined here as persistence of both for 50 years). The likelihood of coexistence is much higher in the stochastic MF model, where contact probabilities are homogeneous, than in the network model. However, this occurs at the lower values of transmissibility only, at the expense of underestimating DFTD

extinction (Fig. 4a and 4b). Both models peak in the likelihood of coexistence at similar low values of transmissibility, and as expected, coexistence probabilities drop to zero as values for transmissibility approach to one. Coexistence is possible in the network model only for the longest latent period estimate (12 months).

A limitation of this study is that our models assume constant values for key disease parameters such as transmissibility and disease induced mortality with each generation of infection. Cancerous tumour cells are, however, usually under strong evolutionary pressure (Merlo *et al.* 2006) and so these parameters could be changing. The potential for coevolution of DFTD and the devil is evident in the ongoing evolution of multiple strains of the tumour (A.M. Pearse, unpublished data), in geographic variation in MHC gene diversity involved in tumour recognition (Siddle *et al.* 2010) coupled with recent data indicating that devil populations in which MHC is variant from the tumour have lower infection rates and longer survival periods with DFTD (Hamede *et al.* submitted), as well as genetic and adaptive phenotypic changes in the devil (Jones *et al.* 2008; Lachish *et al.* 2011). Including tumour strain dynamics and heterogeneities in disease-induced mortality in devil-DFTD epidemic models should help to further investigate effects of virulence in the epidemiology of DFTD.

Implications for disease epidemiology and management

Several conclusions can be drawn that are relevant for the epidemiology and thus management of DFTD and other infectious wildlife diseases. Local extinctions of Tasmanian devil populations affected by DFTD are predicted in a time frame that fluctuates between 5-15 years (dependent on the value of transmissibility used) following disease arrival. This time period is similar to those predicted using both mark-

recapture and stochastic-dynamic modelling (~ 10 years, McCallum *et al.* 2007). To date, no local extinctions from DFTD are known, although as yet the longest known time since disease arrival at a monitored field site is 15 years, (at Mt. William National Park, where the disease was first detected in 1996, Hawkins *et al.* 2006). The decrease in population size at this site has been estimated to be at least 90% (McCallum *et al.* 2007) and devils might have already become locally extinct, without subsequent re-colonization through immigration from adjacent populations. Once population size has been drastically reduced, other important threatening processes such as stochastic environmental and demographic processes or Allee effects can also hasten local extinctions. Given that only the northwest of Tasmania remains free of DFTD and the short time frame for predictions of local extinctions after disease arrival, establishing captive and wild insurance populations must form a critical component of a comprehensive management plan aimed to enhance the conservation prognosis of the species.

Thus far, DFTD has not disappeared from any population after it has become established, even at extremely low population densities (McCallum *et al.* 2009). Moreover, an adaptive management approach to selectively cull all infected individuals has failed to decrease the rate of disease prevalence or to reduce the population-level impacts (Lachish *et al.* 2010). One explanation for the persistence of DFTD at very low population densities is that cyclic periods of high transmission, which could mimic a sexually transmitted disease, might allow DFTD persistence in affected populations. Indeed, age-structured deterministic models have shown that DFTD transmission is consistent with frequency rather than density dependence (McCallum *et al.* 2009), typical of sexually transmitted diseases.

Whether coexistence between DFTD and the devil is the outcome of our models depends to a great extent on the length of the latent period and its interaction with the disease generation time (latent + infectious periods). When the latent period is less than the infectious period (fixed at 6 months in our model) the disease can only persist for high values of transmissibility. On the other hand, when the latent period is greater than the infectious period, the disease can be sustained for a wider range of transmissibility: at high values of transmissibility it brings the host to extinction and at low values of transmissibility achieves coexistence. Obtaining information on the variability of the infectious period across different genotypic populations and strains of DFTD may offer new insights for predicting the likelihood of coexistence.

Conclusions

Determining the epidemic threshold above which disease outbreaks are possible depends not only on the transmission and recovery rates but also on social mixing parameters and the structure of the host population (Volz & Meyers 2009). Measuring contact heterogeneities and social networks in wild populations should become more practical as technological innovations surrounding data collection become available (Krause, Wilson & Croft 2011). The inclusion of observed network properties in our transmission models had a modest effect on the transmissibility threshold for $R_0 > 1$, decreasing it by ~15% compared to a stochastic model with MF assumptions. Given the other uncertainties in estimating R_0 in these wild populations, it is probably unnecessary to obtain further estimates of contact heterogeneities or to include them in models for assessing alternative management strategies. This is unlikely to be the case for many other wildlife diseases (Tompkins *et al.* 2011). Quantifying heterogeneities in contact patterns and using modelling approaches to investigate the implications of these

heterogeneities for disease dynamics is an important step in developing approaches for managing emerging diseases in wildlife populations.

Supplementary Information

Stochastic model structure and algorithm

Figure S1a illustrates the effect of Gaussian parameter V on network connectivity. For values less than 0.003K, short-range distances are strongly preferred (Fig. S1a), leading to a strongly-clustered, disjointed network. Conversely for large values (Fig. S1b) there is no weighting of long-range contacts relative to short-range ones and each node has many neighbours. Figure S2 shows how varying V allows the model to be interpolated from network to mean-field behaviour: for a network with N nodes and mean degree d, the quantity d/(N-1) is seen to approach unity (the mean-field limit) as V increases. Interestingly, once V>0.003K all nodes typically belong to the giant component.

A) B)

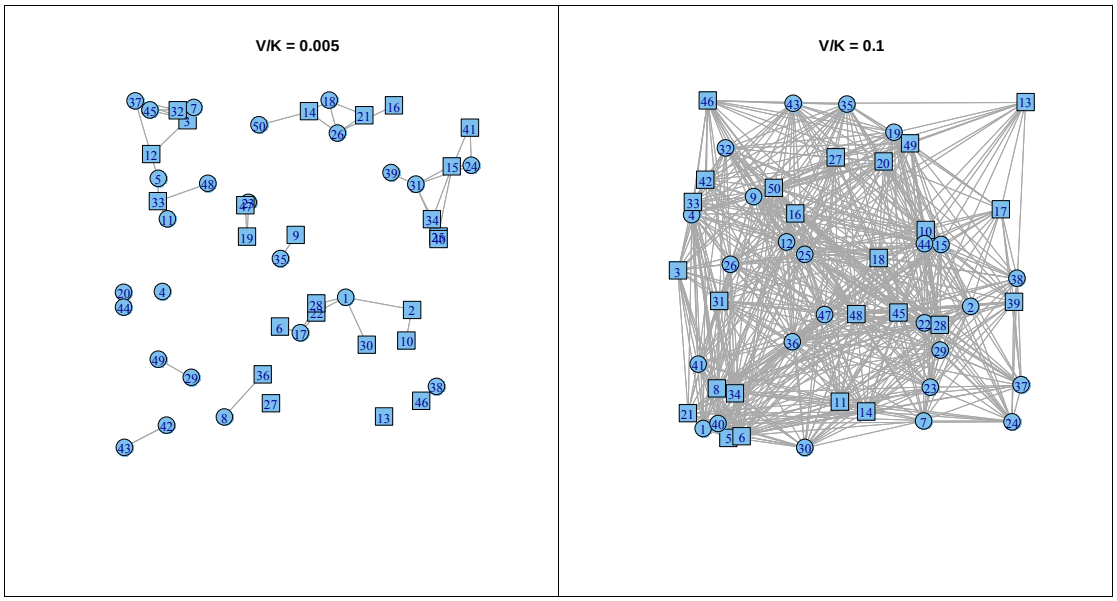


Figure S3.1 A) 50-node network grown using V<0.003K.
B) 50-node network using V>0.003K.

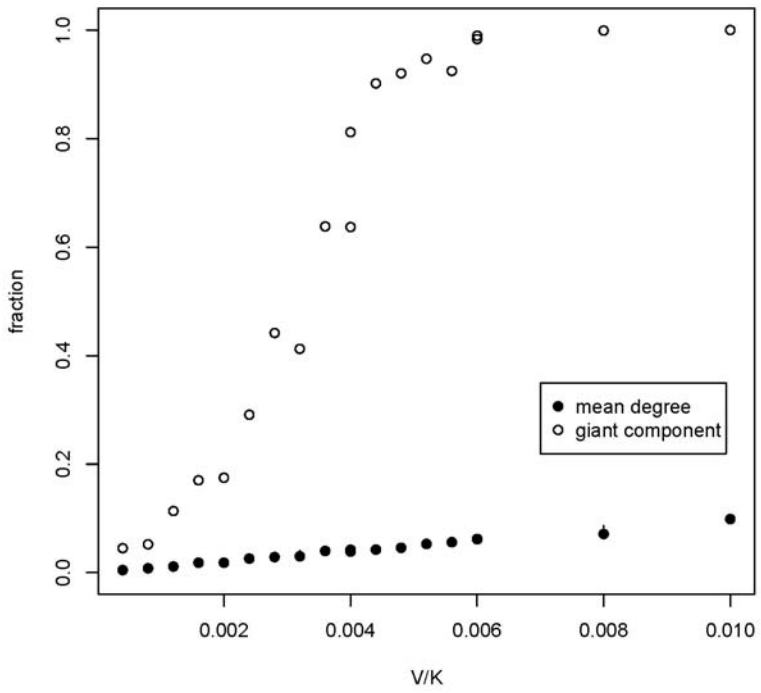


Figure S3.2 Dependence of giant component size and fractional degree (95% CI) upon parameter V, averaged over 500 simulated networks. All other algorithm parameters were kept fixed.

Host population dynamics

At regular time intervals δt_{rec} (assumed here to be annual), new nodes are added to the network, corresponding to the annual 'pulse' of recruitment into the adult population. If the number of females which reproduce in a given year is n_F, the number of recruits in two years time is $n_F R$. Juveniles are recruited into the network model once they reach sexual maturity (2 years of age), as there is no contact data available for this age class. This means that the number of recruits (which will be 2 years old) will depend in the number of reproductive females two years before. We assume the number of recruited males and females to be equal as no study has found significant differences in pouch

young sex ratio (Guiler 1970; Hughes 1982; Pemberton 1990; Lachish et al. 2009). Devils are synchronous annual breeders and females are capable of producing up to four young per litter (Guiler 1970; Pemberton 1990), however the average number of recruits per breeding female in our model is assume to be 2. Slightly higher estimates of litter size, ranging from 2.88 (Guiler 1970) to 3.4 (Lachish et al. 2009), have been reported. However, both estimates have been obtained before pouch young reach independence and therefore do not account for mortality after weaning or dispersal.

The adult devil population is subject to a natural density- and age-dependent mortality. Assuming a Gompertz ageing law, when the total population is *N*, for an animal of age *a*, this rate is

$$\mu = m(1 + \frac{RN}{mK})\exp(Ga)$$

Here R is the average number of annual recruits, per breeding female, into the adult population, which we assume to be 2 (see Table 1 for all parameter values). In the presence of the SEI pathogen this applies to susceptible and exposed individuals. Infected animals experience an additional disease induced mortality μ_I (Fig 1).

Transition Dynamics

For each adult *i*, of age *a*, a time of 'next reaction' is drawn from an exponential Probability Distribution Function (PDF) with an appropriate (according to disease status) rate parameter viz

$$\delta t_i^S = -\frac{\ln p_i}{\mu + \beta},$$

$$\delta t_i^E = -\frac{\ln p_i}{\mu+\lambda},$$

$$\delta t_i^I = -\frac{\ln p_i}{\mu + \mu_I},$$

where the p_i are random uniformly distributed numbers between 0 and 1. In the mean field case disease transmission is determined by rate β, while for the network model the relevant quantity depends on *T*, the probability that a contact transmits the infection and n_I^i, the number of infected neighbours which susceptible *i* has. In this case we make the replacement

$$\beta \to 1-(1-T)^{n_i^I}$$

Network Rewiring

At regular time intervals, δt_{seas}, the network is to be rewired to emulate shifting association preferences between seasons. When a rewiring event occurs, new random numbers p_i are drawn for each animal and the new edges are formed probabilistically, according to the formula Eq. 1 (main text)

For each recruit a random number, p_i is generated, and the network is rewired as described in the preceding step.

The Algorithm

Given these rules, we follow our stochastic algorithm for running each simulation. Statement of the algorithm is:

1. Set the global time t=0
2. Compute, for each animal the time of 'next reaction' using the procedure described in (i) above. Sort these times into an event queue, in order of increasing length. Add multiples of δ_{seas} and δ_{rec} to the event queue, re-sorting if necessary.
3. For steps j=1... take the time at the start of the event queue $t=t_j$ and implement the corresponding event as follows:
   (a) For a rewiring event, the old network edges are deleted, and a new one is grown using edge formula Eq. 1 (main text)
   (b) For a recruitment event, each new animal must be assigned a random number p_i and given edges according to formula Eq. 1 (main text)
   (c) Susceptible and exposed animals have two possible fates: progression to the next stage of disease or death. For example, an exposed animal has a probability of dying given by $\mu/(\lambda+\mu)$. Survival is decided via a Bernoulli trial, using this as the success probability. If the animal survives, its time of next reaction is calculated by generating a random number, p_i as before, and replacing the old interaction time by

$$\delta t_i \rightarrow \delta t_i - \frac{\ln p_i}{\lambda+\mu}$$

An analogous procedure is used to decide the fate of susceptible animals.

(d) If an animal dies, its node is deleted from the contact network.

(e) Re-calculate the event queue and increment the global time $t \rightarrow t+t_j$

4. The simulation is terminated when either host or pathogen goes extinct, or some elapsed global time is reached.

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CHAPTER 4

DFTD in a transition zone: reduced impact of Tasmanian Devil Facial Tumour Disease at the current disease front.

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Abstract

Pathogen-driven declines in animal populations are increasingly regarded as a major conservation issue. The Tasmanian devil (*Sarcophilus harrisii*) is threatened with extinction by devil facial tumour disease, a unique transmissible cancer. The tumour is clonally transmitted in the form of a tissue graft, which is possible because of low host genetic diversity, particularly in the major histocompatibility complex genes of the immune system. The far northwest of Tasmania now holds the last remaining disease-free wild devil populations. The recent discovery of unique major histocompatibility complex genotypes in the northwestern region of Tasmania has raised the possibility that some animals may exhibit resilience to the disease. Here we provide evidence of differences in the epidemiology and population impacts of devil facial tumour disease between three well-studied affected populations in eastern Tasmania and a western population, "West Pencil Pine". In contrast to all three eastern populations, there has been no rapid increase in disease prevalence or evidence of population decline at West Pencil Pine. Moreover, this is the only population in which population age structure has remained unaltered four years after disease arrival. The most plausible explanations for the substantial differences in population impacts and epidemiology of the disease between eastern and western populations are either geographic differences in genotypes or phenotypes of devils or functional differences between tumour strains in the two regions. Future conservation strategies for the species should focus on identifying whether either or both of these explanations is correct and then, if resistance alleles exist, attempting to spread those into already affected populations. Our study highlights the importance of measuring genetic and phenotypic variation in hosts and their pathogens for better understanding disease dynamics and managing their impact on animal populations.

Introduction

Infectious disease is increasingly being recognised as a significant issue in conservation biology (McCallum and Dobson 1995; de Castro and Bolker 2005; Thompson et al. 2010). Diseases that have recently emerged or that have been introduced into naïve populations have particularly severe effects on susceptible host populations (for example, avian malaria in Hawaii [van Riper et al. 1986], amphibian chytrid fungus [Kilpatrick et al. 2010], white nose fungus [Hallam and McCracken 2011] and Tasmanian devil facial tumour disease [McCallum 2008]). Although the outcome of host-pathogen coevolution is complex (Anderson and May 1982; Frank 1996), there is intense selective pressure on the host population to develop resistance to a pathogen with a high mortality rate and there may also be selective pressure against virulent pathogen strains because they kill their host before they successfully transmit.

Strategies for managing disease in threatened animal populations are limited. Options that have been attempted include culling, either of all animals or of infected individuals only (Lachish et al. 2010), vaccination or treatment of infected individuals (Cleaveland 2009), and establishing or maintaining disease-free insurance populations (McCallum and Jones 2006). Attempting to accelerate the process of coevolution between host and pathogen by identifying and spreading host genotypes with some resistance to the disease (Wobeser 2002; McCallum and Jones 2006) is a potential strategy, although we know of no case where this has been applied. Here, we report evidence that a population of Tasmanian devils (*Sarcophilus harrisii*) genetically distinct from other infected populations may have greater resistance to infection with the extinction-threatening devil facial tumour disease.

Until the emergence of devil facial tumour disease, the Tasmanian devil was regarded as common throughout its geographic range. Severe, ongoing population declines caused by the epidemic have led to the devil being listed as endangered at international (IUCN Red List of Threatened Species), national and state levels. The disease, which has now spread to more than 80% of the geographic range of the species (McCallum et al. 2007), is characterized by fast growing tumours, usually in the head and neck region, which often metastasize to the lungs and lymph nodes (Loh et al. 2006). To date, there is no evidence of recovery once clinical signs arise.

The epidemiology and population impacts of the disease have been consistent and unequivocal in all populations for which medium to long-term data are available (3-10 years after disease arrival). Transmission dynamics are characterized by a rapid increase in disease prevalence in sexually mature devils (2 years old and older) (McCallum et al. 2009), and a steady decline in adult survival rates 2-3 years after disease arrival (Lachish et al. 2007), resulting in an immediate and sustained decline in population size and in population growth rate (Lachish et al. 2007, 2010). Population age structure is subsequently drastically changed, with high mortality of adults resulting in a young mean age of the population (Lachish et al. 2009). Reproductive compensation does occur, via a decline in the age of first breeding in females, but is insufficient to mitigate population decline (Jones et al. 2008; Lachish et al. 2009). Age-structured epidemiological models as well as empirical data on social interactions and injury patterns have shown that disease transmission is more consistent with frequency dependence than density dependence (McCallum et al. 2009; Hamede et al. 2008, 2009), which means that there is no threshold host density for disease persistence. Thus this host specific pathogen is capable of driving the Tasmanian devil to extinction (de

Castro and Bolker 2005) and culling to reduce host density is not a viable management strategy.

Devil facial tumour disease is a contagious cancer, in which the tumour cells themselves are the pathogenic agent. Tumours were first detected in 1996, in northeastern Tasmania. The disease has subsequently spread south and west (Hawkins et al. 2006; McCallum et al. 2007). Transmission occurs by direct transfer of tumour cells, most likely when individuals bite each other during social interactions (Pearse and Swift 2006). The establishment and growth of the tumour cell line in a susceptible individual is facilitated by extremely low genetic diversity (Jones et al. 2004), particularly in the major histocompatibility complex of genes, which play a key role in self/nonself recognition (Siddle et al. 2007). All individuals from eastern Tasmania share a functionally identical major histocompatibility complex with the tumour, which originated in an eastern devil (Siddle et al. 2007) allowing tumour transmission without immunological recognition of the tumour as nonself. Some geographical variation in major histocompatibility complex copy number has been found in the more isolated northwest region of Tasmania, raising the possibility that individual devils may have different degrees of susceptibility to the disease (Siddle et al. 2010). These differences in major histocompatibility complex type of individual devils and the tumour may be sufficient to trigger an immune response (Siddle et al. 2010).

In 2006, we established a monitoring site at “West Pencil Pine” which was at that time the furthest extent of the disease in northwestern Tasmania. There is evidence that eastern and western Tasmanian devil populations differ somewhat genetically, both in microsatellites (Jones et al. 2004; Farmer 2006) and major histocompatibility complex

(Siddle et al. 2010). West Pencil Pine is located between eastern and western populations and is the first genotyped population with genetically disparate (and major histocompatibility complex-disparate) animals encountered by the westward spreading disease (Fig. 1). Here, the disease has encountered host genotypes constitutionally different to its own genotype, providing an opportunity to assess the effect of differences in host genetic diversity on disease impacts and transmission dynamics in the wild.

Our objectives in this study were: (i) to determine infection dynamics and temporal changes in disease prevalence within the West Pencil Pine population, (ii) to estimate the impact of devil facial tumour disease on the West Pencil Pine population growth rate, size and demography, and (iii) to compare these results with three affected populations in eastern Tasmania for which consistent medium to long-term data are available from the beginning of the epidemic.

Methods

Study areas and data collection

We analysed data from Tasmanian devil populations at four sites (Fig. 4.1), which were regularly monitored prior to or following devil facial tumour disease arrival. Two sites (West Pencil Pine and Fentonbury) allow robust comparison and interpretation of results because they are of equivalent geographic area, are situated in continuous habitat, and have been sampled using identical time intervals. The other two sites (Freycinet and Forestier) are larger, and are peninsulas with a degree of site isolation.

West Pencil Pine (41° 31' S, 145° 46' E) is a 25km^2 area situated on private production forestry land to the west of Cradle Mountain in northwest Tasmania. This site was established in May 2006 (when the disease was first detected), following three exploratory expeditions aimed at locating the epidemic front. We subsequently sampled this population four times per year, from August 2006 until May 2010, at three month intervals, using 40 traps set for 10 nights. Trapping sessions were undertaken during four seasons coinciding with key life-history events: February (when juveniles are dispersing prior to the mating season), May (immediately after the mating season), August (when females are carrying pouch young) and November (when females are in late lactation).

Fentonbury (42° 37' S, 146° 47' E) is a 25km^2 area situated in the Derwent Valley in southeast Tasmania. Fentonbury was trapped at three monthly intervals from November 2004 until November 2007 using identical trapping protocols to West Pencil Pine. The disease was detected and confirmed for the first time in February 2005.

The Freycinet peninsula (42° 09' S, 148° 17' E) is 160km^2 in area, extending approximately 30km from North to South and has been monitored since July 1999. In order to use comparable time frames in all sites, the data set we used in this paper was derived from trapping trips undertaken from June 2001 (when devil facial tumour disease was first confirmed) until 2006. We used data from April and July trips, which were conducted in most years (except 2003 and 2004). Detailed site descriptions and trapping protocol are given in Lachish et al. (2007).

The Forestier peninsula (42^{o} 56' S, 147^{o} 53' E) is a 70km^{2} area that has been monitored since July 2004, at first detection of the disease and when prevalence was very low (1.5%). The data set we used in this paper is derived from four trapping trips per year (40-50 traps set over 10 night trapping sessions), carried out in February, May, August and November, between November of 2004 and 2006 and five trapping sessions (an extra trip in June) from February 2007 until June 2008. All animals with signs of disease have been removed from the population at the time of capture in a disease suppression trial (Lachish et al. 2010).

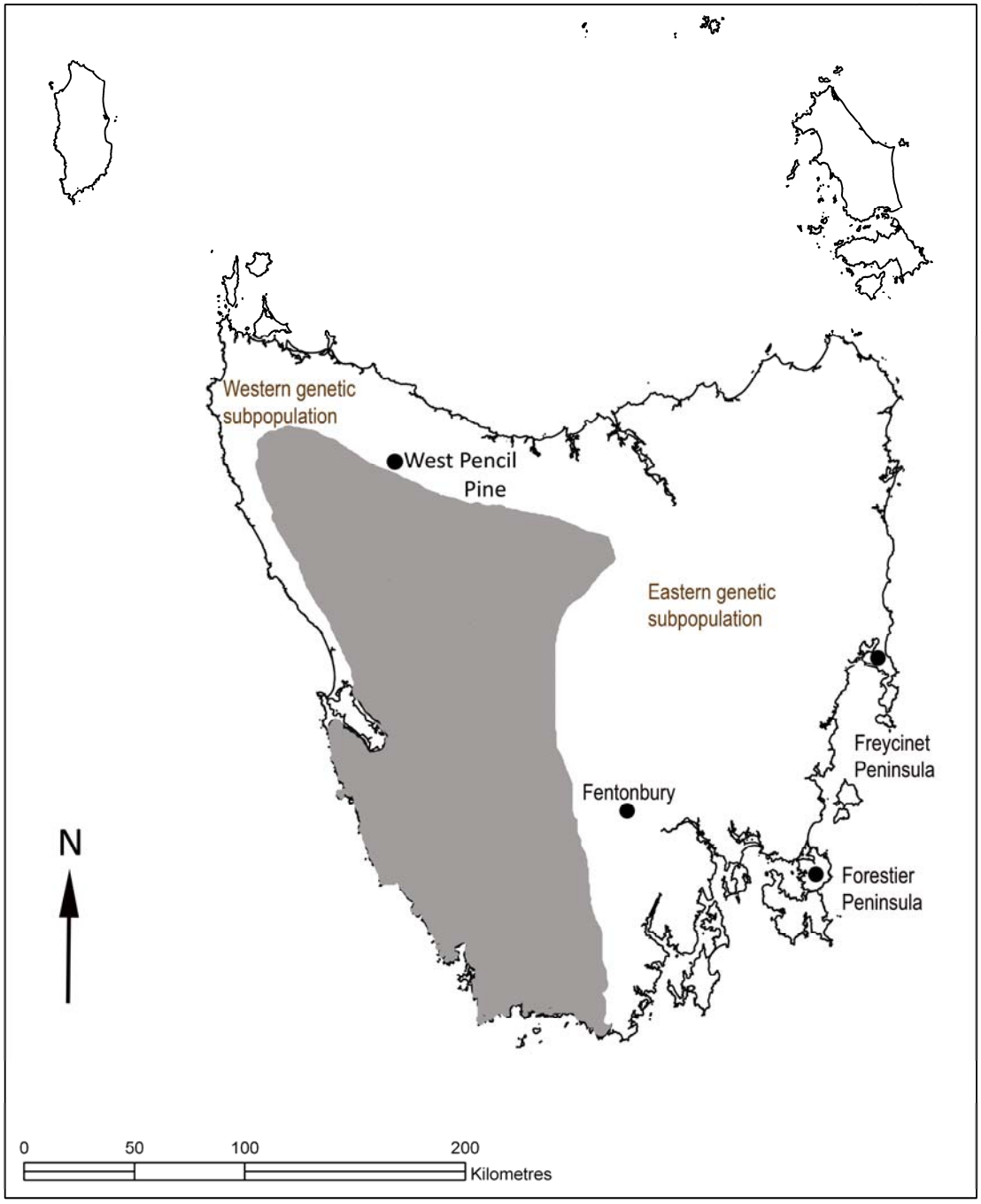

Figure 4.1 Map of Tasmania indicating the locations of the study sites (black dots), the two genetic subpopulations and the transition zone (Jones *et al.* 2004; Farmer 2006). The grey shaded area corresponds to low devil densities due to unsuitable habitat (Jones & Rose 1996).

Identification of individuals, disease and age classes

All captured devils were identified with unique microchip transponders (Allfex, New Zealand) with the exception of the Freycinet Peninsula where unique ear tattoos were used prior to 2004. Disease status was assessed by histopathological examination of biopsies from tumours (Loh et al. 2006), or when this was not possible, by visual inspection and identification of tumours (see Hawkins et al. 2006 for visual detection methods). In our data set we have only categorized individuals as diseased if confirmed by histopathology analyses or if visually scored as definitively having tumours (Hawkins et al. 2006).

Because all sites have been monitored regularly, most of the individuals in our data set were originally captured as subadults and are therefore of known age. We aged devils first captured as adults using a combination of molar eruption, molar tooth wear and canine over eruption (distance from dentine-enamel junction to the gum; M.J, unpublished). This method is considered precise for ageing devils up to 3 years of age (M.J., unpublished data). In some analyses, devils with estimated ages of three years and older were pooled into a single age class. Older devils of known age were used for age structure analyses. As Tasmanian devils are seasonal breeders (breeding season between March-May), we used the mean annual birth date of March 20th for allocating age classes.

Statistical analyses

Changes in disease prevalence following disease arrival, as a function of age class and site, were initially analysed with logistic models in R (version 2.9.0, R Development Core Team 2007) but residual deviance suggested overdispersion in the data.

Subsequently, we used Generalized Linear Mixed Models implemented in the function "lmer" in R package lme4, with the interaction of site, trapping session and age class included as a random term. Although we have visually presented data from all four sites, we have mostly restricted statistical analysis to comparisons of West Pencil Pine and Fentonbury only, because the elongated linear configuration of Freycinet confounds disease spatial spread and increase in prevalence through time (McCallum et al. 2009) and because diseased animals were removed at Forestier. Preferred models were selected on the basis of small sample corrected Akaike Information Criteria (AICc) and Akaike weights (Burnham and Anderson 2002).

Changes in age structure among the four sites were analysed by modeling the proportion of less than 3 year old devils versus older than three years old devils in each population. Devils live up to six years in the wild. We used generalized linear models with binomial error distribution, with season and site as factors and time since disease emergence as a continuous predictor variables and allowing all possible interactions between covariate predictors. Recently weaned devils (<1 year old) were excluded from this analysis as this age class only starts to enter the trappable population consistently in the weeks prior to weaning.

Capture-mark recapture data were analysed using the program MARK (Cooch and White 2002). Size of the adult component of the population was estimated using the POPAN open population models available in MARK. Population estimates were obtained from models with constant recapture rates, and time-variation in survival rates and probability of entry (PENT) parameters. The February trapping sessions were excluded from these analyses as the large cohort of newly-independent juveniles

influences the success of capturing adults at this time of year. Differences in population size changes among sites were investigated using linear regression with season as a factor, trend (time since disease arrival) and site as predictor variables, weighted by the inverse of the squared standard error of estimated population size.

To compare realized finite population growth rates (λ) at Fentonbury and West Pencil Pine following disease arrival, we used reverse-time (Pradel) models (Pradel 1996). Pradel models do not assume a stable age distribution and do not allow age class effects. We therefore restricted our models to the adult (2+ years) component of capture histories. As there is an annual pulse of recruitment into the adult population each austral summer, we calculated growth rates from yearly population estimate intervals. We used data from May trapping trips at West Pencil Pine and from November trips at Fentonbury as these were the trapping trips that were conducted consistently throughout the entire sampling period. As recruitment does not occur between May and November, between site comparisons using data collected at these different times of the year are possible. We used a Goodness of Fit (GOF) parametric bootstrapping test (White and Burnham 1999) to test for over-dispersion in the data. After estimating the best supported model for survival (based on AICc adjusted for over-dispersion QAICc; Burnham and Anderson 2002), we tested the effect of time since disease arrival and disease prevalence on population growth rates.

Results

Changes in disease prevalence following disease arrival

Disease prevalence remained relatively low (20%) compared to older animals in 1-2 year old devils at all sites throughout the sampling period (Fig. 4.2a,b,c and d) but increased rapidly (to between 40 and 80%) in 2-3 year old devils in all populations, except for West Pencil Pine, where prevalence remained below 20% (Fig. 4.2e,f,g,and h). Prevalence in devils older than 3 years old also increased much more rapidly at Fentonbury, Freycinet and Forestier than at West Pencil Pine (Fig. 4.2i,j,k and l).

The best supported model from our West Pencil Pine -Fentonbury analysis included all possible two-way interactions between age class, site and trend, indicating that the rate of increase in disease prevalence differed both between age classes and sites (Table S4.1). When age classes are analysed separately, models including a site*trend interaction were very strongly supported for each age class (Akaike weight 0.81 for 1-2 year olds, 0.95 for 2-3 year olds and 0.99 for 2-3 year olds), due to a much more rapid increase through time in disease prevalence at Fentonbury than at West Pencil Pine (site:trend interaction parameter estimate for 1-2 year olds = 3.2 [SE 2.01]; for 2-3 year olds = 1.55 [SE 0.54]; for 3+ year olds = 1.55 [SE 0.45], all for Fentonbury relative to West Pencil Pine).

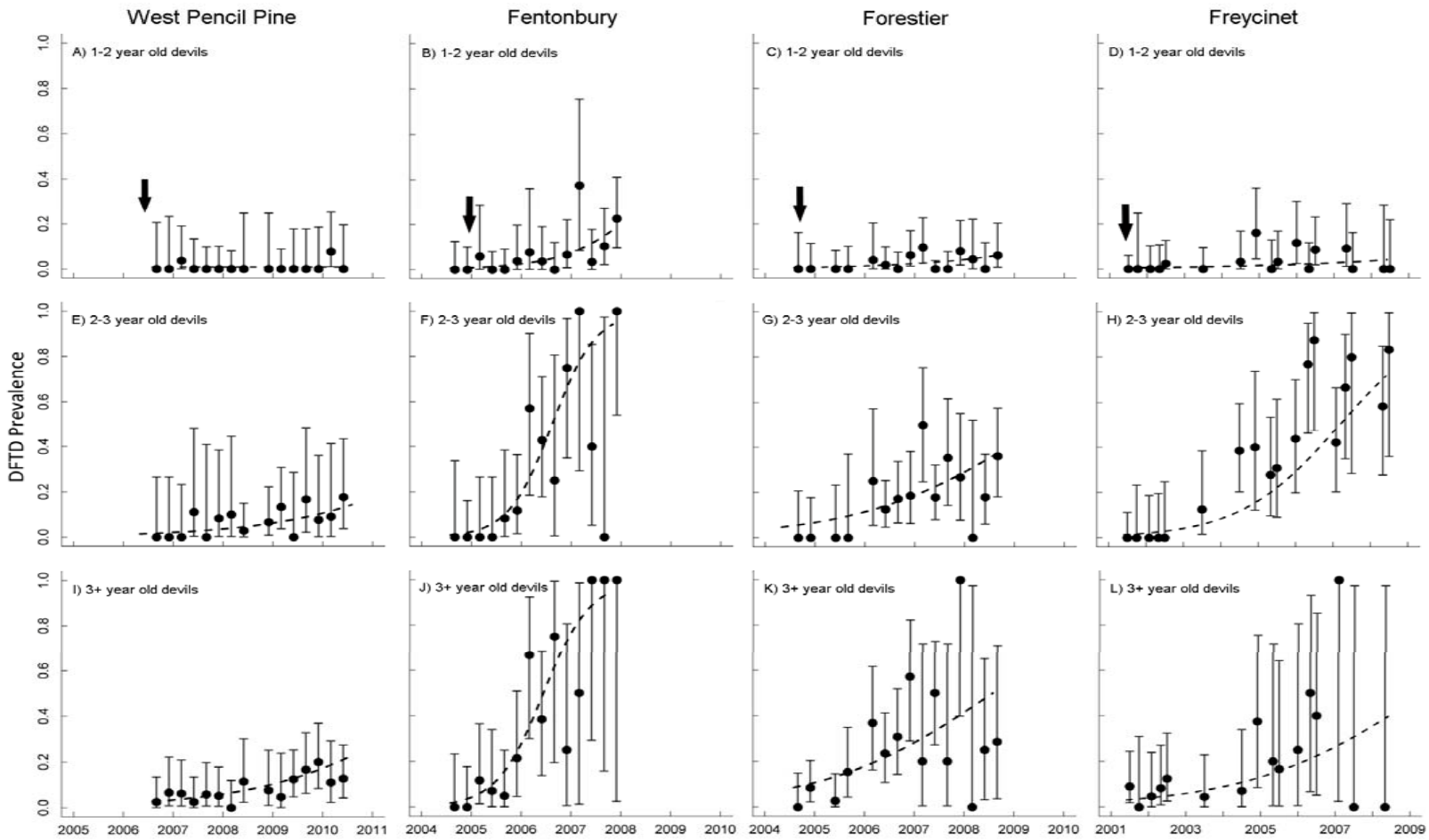

Figure 4.2 Prevalence of devil facial tumour disease at four different sites that were monitored shortly after disease arrival: West Pencil Pine (A, E and I), Fentonbury (B,F and J), Forestier (C, G and K) and Freycinet (D, H and L). Prevalence was estimated in three different age classes: 1-2 2, 2-3 and 3+ years old. Error bars are exact binomial confidence intervals and the dashed line represent the best fit of a logistic regression model. The black arrow indicates the time at which devil facial tumour disease was first detected at each population.

Changes in population age structure

Individuals older than 3 years of age (and particularly devils aged 4+ years) virtually disappeared from all sites except West Pencil Pine, resulting in populations composed of mainly 1 and 2 year old individuals. In contrast, individuals of all age classes remained in relatively similar proportions at West Pencil Pine during the entire sampling period (Fig. 4.3). The proportion of individuals in age classes < 3 years vs > 3 years did not change significantly with time since disease arrival at West Pencil Pine ($p = 0.842$). Relative to West Pencil Pine there was a significant increase with time since disease arrival in the proportion of younger animals at all other sites (Fentonbury, $p < 0.000$; Freycinet $p < 0.000$; Forestier $p < 0.000$: see table S4.2 for model parameter estimates).

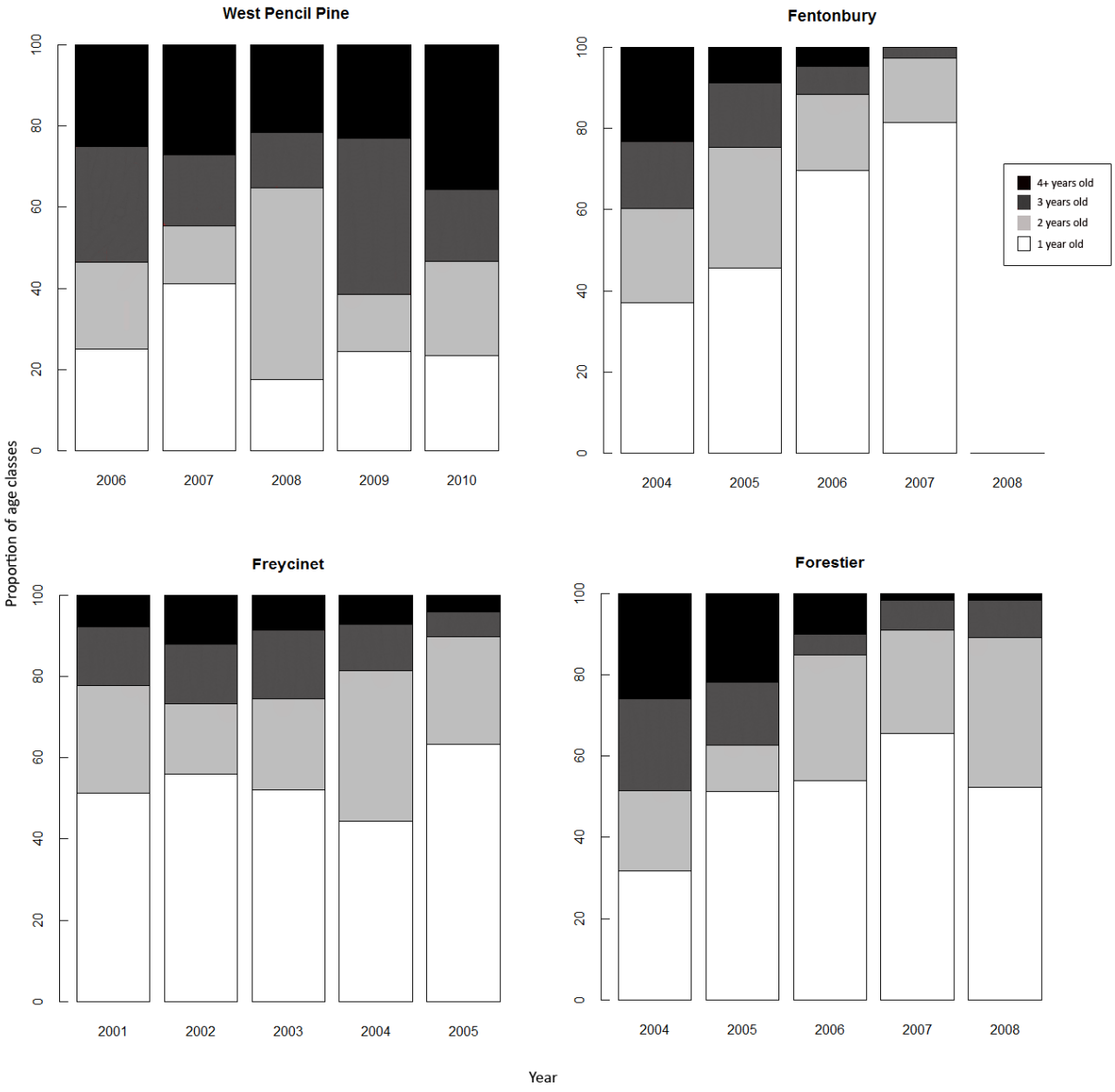

Figure 4.3 Proportion of Tasmanian devils in each of four age classes during winter (July-August) field trips at four sites: West Pencil Pine, Fentonbury, Freycinet and Forestier.

Changes in population size and population growth rate after disease arrival

The adult population size declined following disease arrival at all sites except West Pencil Pine, where population size remained stable and unaltered ($p = 0.577$) four years after disease arrival (Fig. 4.4 and Table S4.3). The final model for the weighted regression included a site*trend interaction, indicating the different pattern through time at West Pencil Pine.

The GOF for the Pradel global model shows no issues of lack of fit of the data ($p = 0.143$) and slight over dispersion ($\hat{c} = 1.44$). The best supported model for apparent survival rates in the adult segment of the population indicates that survival varies through time and between sites in an additive manner (Table S4.4). Population growth rate estimates from Pradel reverse time models comparing Fentonbury and West Pencil Pine showed that, whereas significant population decline occurred at Fentonbury within one year of disease arrival and thereafter accelerated, the population decline was significantly slower at West Pencil Pine and not significantly different from zero three years after disease arrival (Fig. 4.5, Table S4.4).

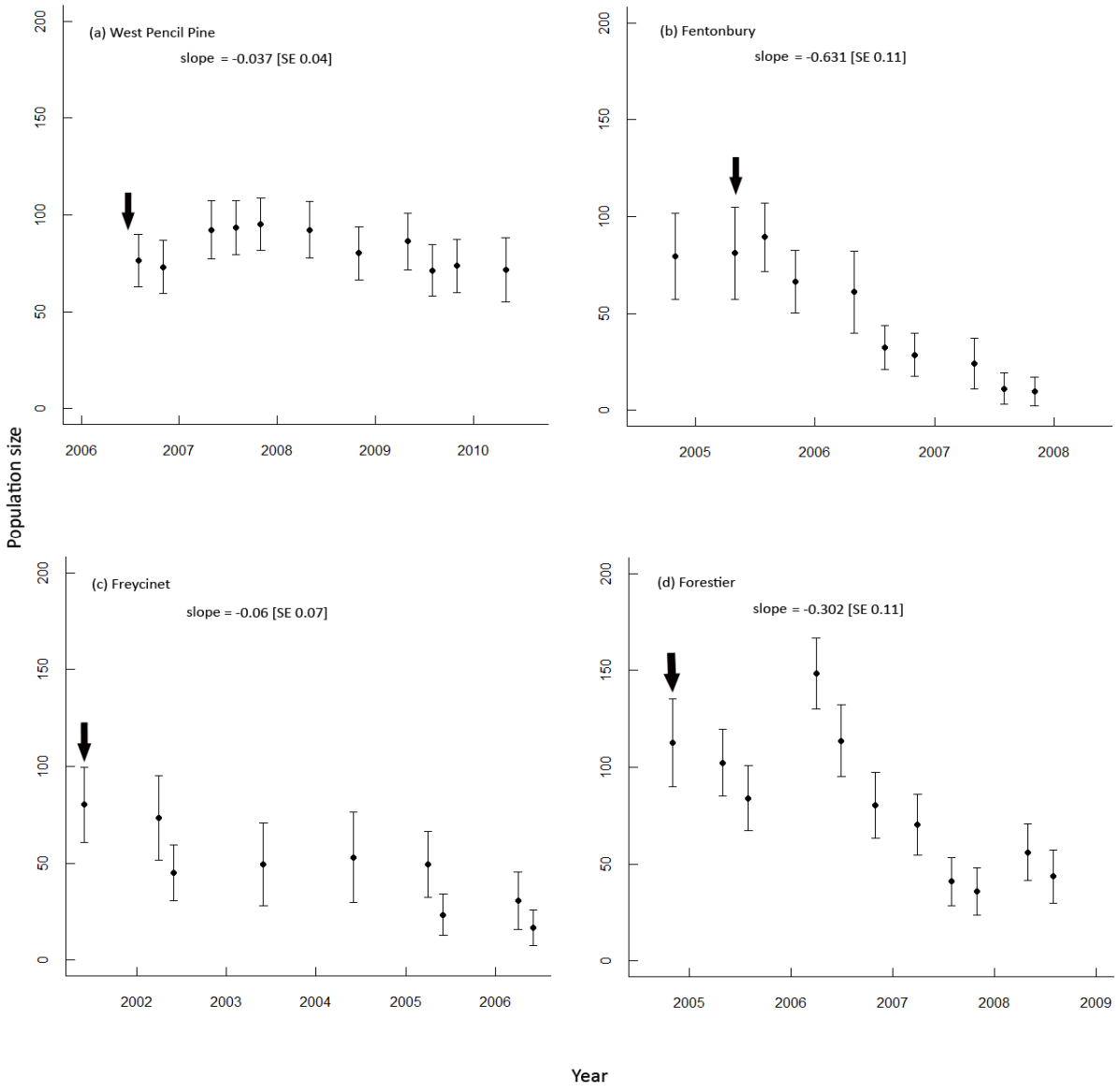

Figure 4.4 POPAN open population size estimates and the slope parameter estimate for the decline at the four sites: A) West pencil Pine, B) Fentonbury, C Freycinet and D) Forestier. Estimates were obtained from the beginning of the epidemic outbreak (first detection of devil facial tumour disease) to up to five years after disease arrival and correspond to the adult segment of the population only. The black arrow indicates the time at which devil facial tumour disease was first detected at each population. Error bars are 95% CI.

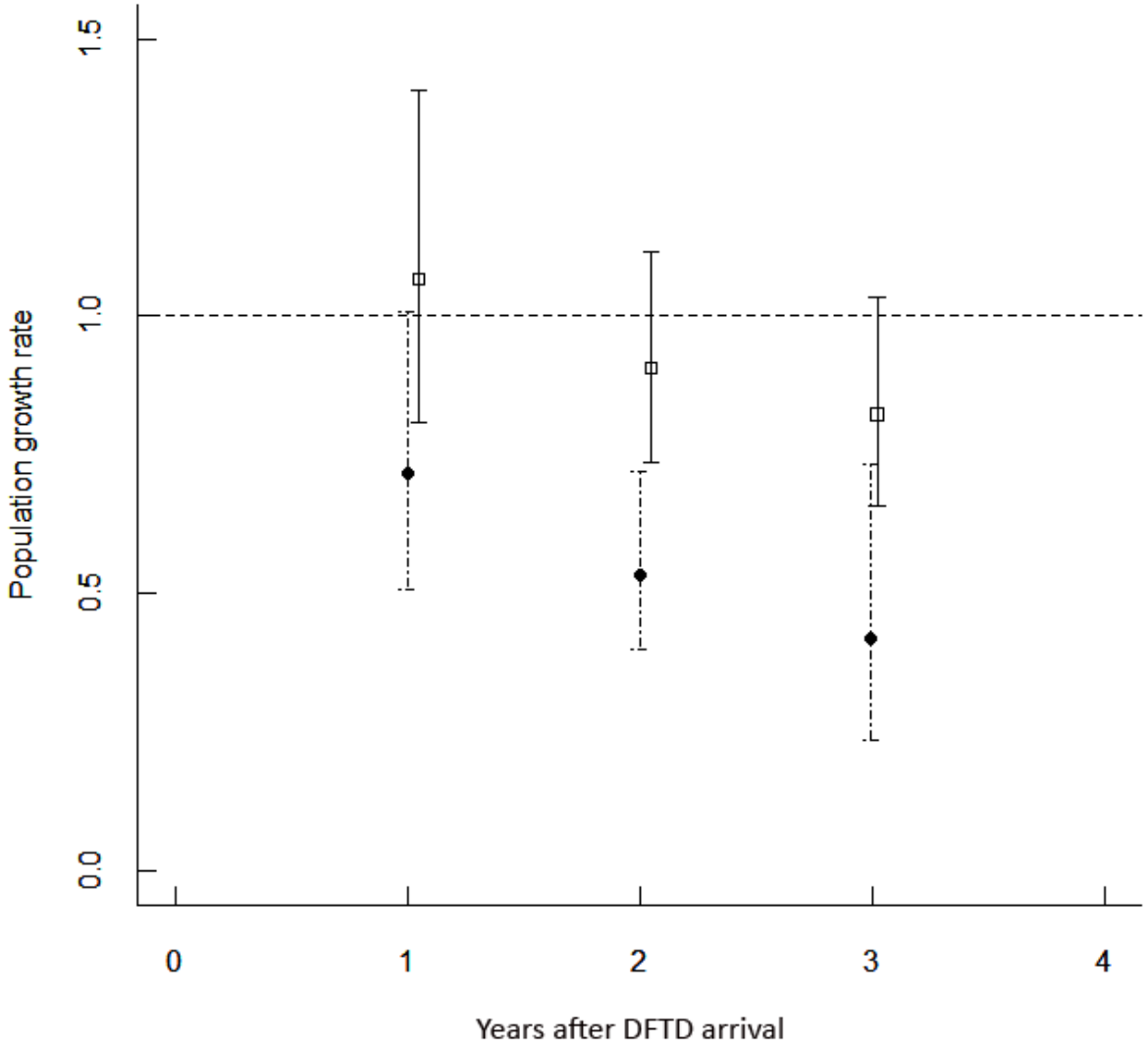

Figure 4.5 Population growth rate estimates from the Pradel reverse time population growth rate models at West Pencil Pine (open squares) and Fentonbury (closed circles). Parameter estimates are model–averaged means from Table S4.4

Discussion

Progress of the epidemic and its impacts on the devil population are markedly different at the West Pencil Pine site, where for the first time devil facial tumour disease is encountering individuals that differ to the tumour in their major histocompatibility complex genotypes (Siddle et al. 2010). In contrast to the observed impacts at all three disease-affected eastern populations, there was no evidence for a rapid increase in disease prevalence at West Pencil Pine in any age class, nor any indication of changes to population size or age structure or significant declines in population growth rate. All eastern populations, in which the major histocompatibility complex genotype of the host matches exactly that of the tumour, exhibited rapid declines in population size following disease arrival (Fig 4.4). Although disease-induced population decline at the Freycinet Peninsula site appeared to occur more slowly than at other eastern populations, this site is considerably larger and also linear in configuration. After its arrival at the northern end of the peninsula in 2001, the disease progressed southwards at ~7km per year, not reaching the southern end of the peninsula until late 2005 (Lachish et al. 2010). Trends in disease impact through time at this site are therefore influenced by disease spread through space.

Likewise, observed impacts of the disease at Forestier should also be interpreted with caution, as this site has been subject to intense management involving the removal of infected individuals. However, a recent assessment of this management operation revealed that selective culling has not altered the course of disease in this population with culling mortality simply substituting for disease-induced mortality (Lachish et al. 2010).

An obvious limitation of this study is that we were able to compare rigorously only one site with non-eastern genetic structure (West Pencil Pine) with one site with eastern genetic structure (Fentonbury) (Jones et al. 2004; Farmer 2006). This limitation was imposed because monitoring sites other than West Pencil Pine were not set up at the time to test hypotheses concerning disease epidemiology and host genetic structure. In principle, any number of factors affecting contact rates between susceptible and infected devils and the probability of infection given contact, including population density and habitat structure, could influence the rate of increase of disease prevalence and impact on the host population. However, in all sites other than West Pencil Pine in which disease prevalence has been monitored (the three sites in this study and four further sites analysed in McCallum et al. 2009), prevalence in 2-3 year olds is at least 50% if the disease has been present for more than three years and age structure has been drastically affected (Jones et al. 2008). This is the case across a variety of habitat types and even if there has been a greater than 90% reduction in population density, as is the case at Mt. William where the disease was first recorded (McCallum et al. 2009). There does appear to be something very unusual about the disease progression at West Pencil Pine, where disease prevalence in 2-3 year olds remains less than 20% and the age structure remains typical of an uninfected population.

There are several plausible explanations for the lack of population impacts and low disease prevalence at West Pencil Pine. One explanation relates to variation in major histocompatibility complex sequence between host and tumour. Typically, major histocompatibility complex diversity is the result of sequence variation within the peptide binding regions of the major histocompatibility complex molecules (Brown et al. 1993). In devils, overall sequence diversity in the peptide binding regions is low with

the majority of unique alleles found in the northwestern populations (Siddle et al. 2010). In addition, variation between devils also occurs in major histocompatibility complex gene copy number, with between 2 and 7 Class I alleles per individual (Siddle et al. 2010). It is conceivable that individuals with a different combination of major histocompatibility complex molecules to the tumour could recognize foreign antigens on the tumour and mount an immune response. These individuals might be less likely to acquire devil facial tumour disease or may show evidence of an immune response (Siddle et al. 2010). Two wild-caught individuals in the West Pencil Pine population have been identified as possessing antibodies to devil facial tumour disease, suggesting that these devils were exposed to the disease and produced a protective immune response (A.K., unpublished data).

A study into association between MHC-type and disease susceptibility in West Pencil Pine is currently in progress. At this stage it is not possible to assign alleles to loci due to high levels of similarity between alleles, making prediction of inheritance patterns of MHC alleles in devils impossible. The genomic organization of the devil major histocompatibility complex is currently being elucidated by sequencing large insert clones. Further immunogenetic research will be critical for elucidating the relationship between major histocompatibility complex diversity and resistance to devil facial tumour disease.

Another plausible explanation for the absence of population impacts of devil facial tumour disease in West Pencil Pine is that the tumour could be evolving into a less aggressive form. Host-pathogen systems are expected to evolve towards increased resistance in the host and optimal pathogen virulence – a pathogen that allows its host to

live long enough for maximal transmission. Such evolution is believed to have occurred in the canine transmissible venereal tumour (Murchison 2009; Belov 2010) a process which led to this tumour becoming the ultimate parasite – highly contagious but not lethal. Compared to the canine tumour, the devil's tumour is still young, but at least eight strains of devil facial tumour disease based on karyotype have been detected (A.M.P., unpublished data). The tumour strain seen in West Pencil Pine differs from the strains seen in the east because it is tetraploid (A.M.P., unpublished data). Tetraploid cells usually grow more slowly and have difficulty undergoing mitosis, and hence have lower rates of proliferation than diploid cells (Margolis et al. 2003; Ganem and Pellman 2007; Storchova and Kuffer 2008). Furthermore, it has been proposed that an active mechanism called 'tetraploid checkpoint' prevents proliferation of tetraploid cells in normal tissues (Margolis et al. 2003). Tetraploidy therefore, may lead to a less aggressive form of the disease. This is supported by preliminary results which indicate that most diseased devils at West Pencil Pine do not succumb to infection as quickly as in Fentonbury and persist for longer periods within the population (see Figure S4.1).

A final alternative explanation for the lack of population impacts at West Pencil Pine is that the low infection rates and slower rate of disease progression at this site could be due to behavioural factors, such as a lower biting rate. The primary route of transmission of devil facial tumour disease is through biting, although transmission via fomites (as devils scavenge on carcasses foraged by diseased devils or directly on infected devils that have succumbed to the disease) cannot be discounted. Contact rates at carcasses are positively associated with increasing population density (Hamede et al. 2008). Population density is higher at West Pencil Pine and Forestier (>2 devils/km^2) than at Fentonbury and Freycinet (<1 devil/km^2) and therefore it is unlikely that the low

infection rates at West Pencil Pine are due to low contact rates. However, other mechanisms such as habitat type or use, heterogeneities in the distribution of food resources across the landscape, or population structure could influence the rate of contact between populations. Biting behaviour is worthy of further investigation in northwest Tasmania.

Options for managing devil facial tumour disease in wild populations are very limited (McCallum and Jones 2006; Jones et al. 2007). Reintroduction to the wild of healthy devils from insurance populations following local extinctions is a viable, albeit long-term management strategy. Broad-scale vaccination of wild populations is a plausible but unlikely option that could be useful only with select captive individuals. Selective culling of infected individuals failed to eliminate the disease at the Forestier population (Lachish et al. 2010). The detection and assisted spread of resistant alleles, if they exist, appears to be the most feasible option for securing devil populations in the wild at a landscape scale. With 100% mortality of infected devils within 6-12 months, selective pressure on devils for any traits that may confer individual disease or population resilience is extremely strong. The different patterns of disease progression and demographic impact that we are seeing in northwest Tasmania, combined with the detection of devil facial tumour disease antibodies at West Pencil Pine and knowledge of the genetic population structure of devils at both neutral and the functional markers involved in tumour recognition, provide some hope that there may be some natural genetically-based resilience to the disease. If resilient populations could be established in parts of eastern Tasmania by translocating resistant animals, these could then act as source populations to repopulate currently decimated eastern populations.

Our study provides tantalizing evidence that differences in host genetic structure or tumour strain may be affecting the progression of devil facial tumour disease, with the possibility of this leading to effective management strategies. Further long term work is clearly needed. To validate the generality of the results reported here, regular monitoring of several populations on the epidemic front as the disease spreads into the genetically divergent populations in northwestern Tasmania should now become be a priority. This will also offer further research opportunities for assessing the immunogenetic responses of western populations to devil facial tumour disease. Our study highlights the critical role of longitudinal studies and the importance of consistent monitoring of natural disease progression in wildlife populations if emerging disease threats are to be addressed.

Supplementary Information

Table S4.1 Results of generalised mixed models comparing the rate of increase in DFTD prevalence at West Pencil Pine and Fentonbury as a function of site, age class and trend.

Competing models	***k***	**AICc**	**ΔAICc**	w_i
All age classes				
(ageclass*trend) + site	7	119.2	23.16	<0.00
site + ageclass + trend	5	117.3	21.26	<0.00
ageclass*site*trend	12	99.12	3.08	0.17
(ageclass*site) + (ageclass*trend) + (trend*site)	10	96.04	0.00	0.82
1-2 year olds				
trend	2	43.38	12.8	<0.00
site	2	39.19	8.61	0.01
site + trend	3	33.73	3.15	0.17
site*trend	4	30.58	0.00	0.81
2-3 year olds				
trend	2	89.85	50.58	<0.00
site	2	64.57	25.3	<0.00
site + trend	3	45.34	6.07	0.04
site*trend	4	39.27	0.00	0.95
3+ year olds				
site	2	68.01	34.75	<0.00
trend	2	63.84	30.58	<0.00
site + trend	3	45.96	12.7	<0.00
site*trend	4	33.26	0.00	0.99

Notes: trend is considered as time since disease arrival, "+" represents an additive effect only whereas "*" represents main effects and interactions, "*k*" represents the number of parameters in each model and "w_i" represents the Akaike weight of each plausible model relative to all the models fitted for that analysis (Burnham & Anderson 2004). All possible models including up to two-way interactions were fitted to the *all age classes* analysis but only models with values <25 ΔAICc are shown. Models for each analysis are shown in order of decreasing values of AICc (small-sample corrected Akaike Information Criterion).

Table S4.2 Parameter estimate values of the generalized linear model for changes in the proportion of 1-2 vs 3-4+ year old devils at each population. Sites effects are relative to WPP.

Parameter	Estimate	SE	P
Intercept	0.036	0.182	0.842
Fentonbury:trend	0.689	0.146	<0.000
Freycinet:trend	0.290	0.074	<0.000
Forestier:trend	0.728	0.104	<0.000

Table S4.3 Parameter estimate values of the linear model for changes in population size (POPAN) after DFTD arrival. Sites effects are relative to WPP.

Parameter	Estimate	SE	P
Intercept	4.512	0.186	<0.000
trend	-0.038	0.068	0.577
Fentonbury:trend	-0.668	0.101	<0.000
Freycinet:trend	-0.063	0.097	0.518
Forestier:trend	-0.265	0.101	0.013

Table S4.4 Summary of the Pradel population growth rate models for apparent survival (φ) and realized population growth rates (λ) at Fentonbury and WPP.

	φ	**p**	**λ**	**ΔQAICc**	**w_i**	**k**	**Deviance**
Stage 1 modeling survival	t	s	s*t	26.66	<0.000	11	44.73
	s+t	s	s*t	0.00	0.999	12	15.93
Stage 2 population growth rate	s+t	s	time	16.89	<0.000	9	35.40
			$fent^{time}$ /wpp^{prev}	4.09	0.038	11	18.36
			site*time	3.79	0.045	12	15.93
			site*prev	1.97	0.112	10	18.36
			site	1.94	0.114	8	22.53
			$fent^{prev}$/wpp^{time}	1.76	0.124	11	16.03
			site + time	0.26	0.263	10	16.65
			site + _prev	0.00	0.301	9	18.51

Notes: Models are shown in decreasing value of ΔQAICc. A forward slash was used to separate the sites when model structure differed at each site. "+" represent additive effects only whereas "*" represents main effects and interaction, "p" = recapture, "k" = number of parameters, "s" = site, "prev" = disease prevalence effect and "t" = time from disease arrival.
The global model was set to allow for site and time variation in survival (φ) and population growth rate (λ) parameters. Recapture rates (p) at WPP were relatively high (0.8) but could not be estimated at Fentonbury as the data were too sparse to reliably fit a model. We therefore evaluated a range of plausible values for p (ranging from very low to very high: 0.3-0.9), for the Fentonbury site data to investigate their effect in the model selection process for estimating φ and λ. The selection and order of the best supported models did not change with any of the range of values for p. We therefore, fixed p at Fentonbury with the same estimate as in WPP.

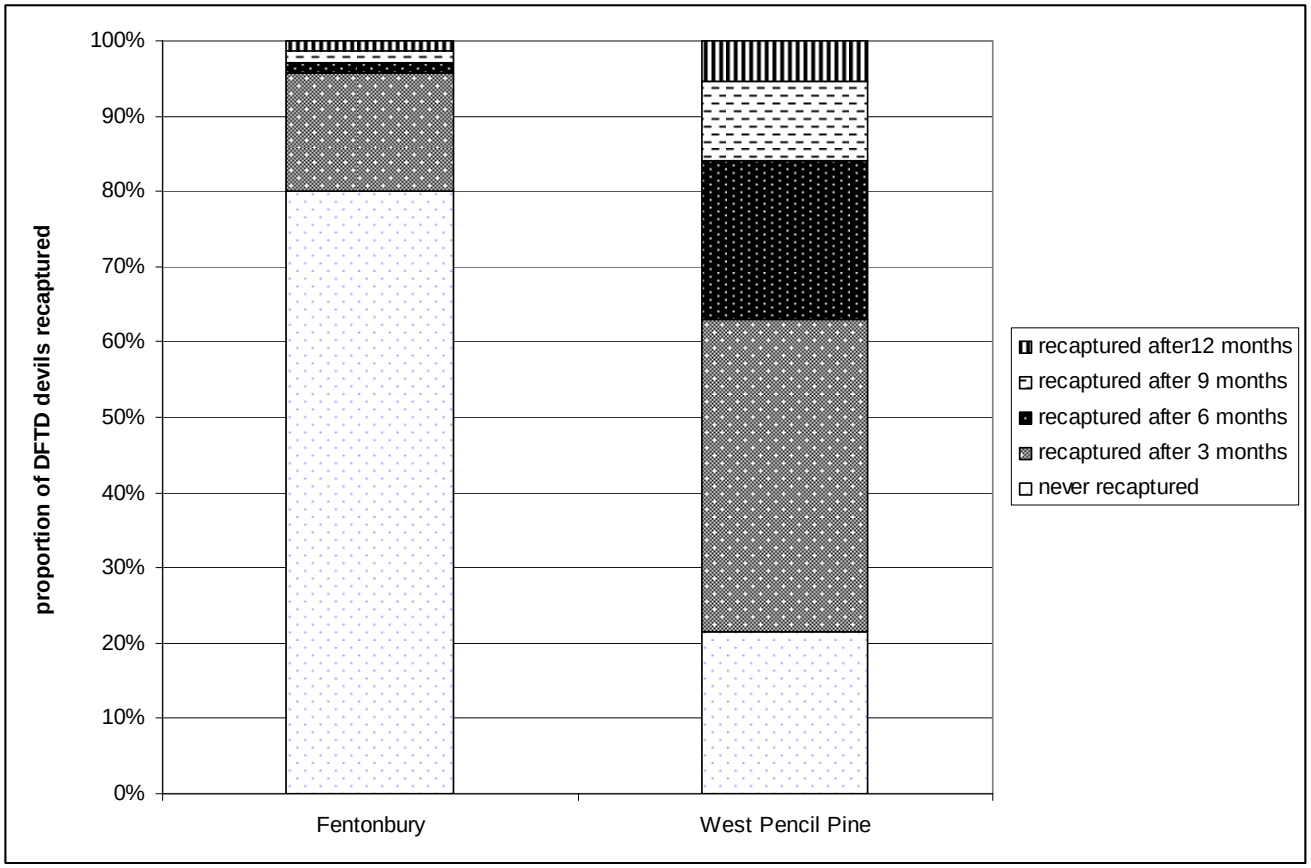

Figure S4.1 The proportion of DFTD devils that survived and were recaptured with DFTD at each site where regular 3 months trapping intervals were undertaken.

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CHAPTER 5

Patterns of biting injuries and transmission of Tasmanian devil facial tumour disease.

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Abstract

The Tasmanian devil is threatened with extinction by Devil Facial Tumour Disease (DFTD), a unique infectious cancer in which the tumour cells themselves, which derive from a single long-dead host devil, are the infective agent and the tumour is an infectious parasitic cell line. Transmission is thought to occur via direct inoculation of tumour cells when susceptible and infected individuals bite each other or by fomitic transfer of tumour cells. The nature of transmission and the extent to which biting behaviour and devil ecology is associated with infection risk remains unclear. Until our recent study in northwest Tasmania showed reduced population and individual impacts, DFTD had caused massive population declines in all populations monitored. In this paper, we investigate seasonal patterns of injuries resulting from bites between individuals, DFTD infection status and tumour location in two populations to determine whether the number of bites predicts the acquisition of DFTD and to explore the possibility that the reduced impacts of DFTD in northwest Tasmanian are due to reduced bite rates.

Devils with fewer bites were more likely to develop DFTD and primary tumours occurred predominantly inside the oral cavity. These results are not consistent with transmission occurring from the biter to the bitten animal but suggest that dominant individuals delivering bites, possibly by biting the tumours of other devils, are at higher risk of acquiring infection than submissive individuals receiving bites. Bite rates, which were higher during autumn and winter, did not differ between sites, suggesting that the reduced population impacts in northwest Tasmania cannot be explained by lower bite rates. Our study emphasises the importance of longitudinal studies of individually marked animals for understanding the ecology and transmission dynamics of infectious diseases and parasites in wild populations

Introduction

Parasites and pathogens are ubiquitous in nature and are an important source of reduction in fitness in wild populations (Hudson *et al.* 2002). The key process in any host-parasite interaction is transmission, but understanding the dynamics of transmission in a wild population is often very difficult (McCallum, Barlow & Hone 2001; Tompkins *et al.* 2011). Inferring how parasite or pathogen transmission occurs in the field and determining the major routes or host behaviours that facilitate or limit disease transmission in wild animal populations is usually hampered by the difficulties of gathering empirical data on actual transmission events.

In a directly transmitted parasite or pathogen, transmission requires sufficient contact between an infected and a susceptible host to enable infective stages to pass from one to another. Host behaviour therefore plays an essential part in pathogen transmission and pathogens may in turn influence host behaviours responsible for transmission (Hart, 1988; Loehle 1995; Wendland *et al*. 2010). For example, sick animals affected by pathogens may become lethargic, seek isolation, avoid contact with conspecifics (Hart 1990; Loehle 1995) or increase aggressive behaviour (Knobel & du Toit 2003). These pathogen-driven responses can alter the contact rates of host populations and alter the dynamics of infection (Artois *et al*. 1991). Theoretical and empirical studies suggest that behavioural changes due to parasites are usually adaptive and beneficial to the parasite rather than to the host (Dobson 1988; Hart 1990). Behavioural differences at the individual level are nonetheless, common in wild populations and evolutionary theory predicts that there should be fitness advantages for susceptible individuals that are capable of recognizing infectious conspecifics and avoiding parasite transmission (O'Donnell 1997; Boots *et al*. 2009).

Tasmanian devils (*Sarcophilus harrisii*) are threatened with extinction by Devil Facial Tumour Disease (DFTD) (Hawkins *et al*. 2006; McCallum *et al*. 2009), a directly transmissible cancer in which live tumour cells are transmitted directly between hosts through intimate contact. Transmissible cancers are a novel type of parasite in the spectrum described by Lafferty & Kuris (2002). They consist of cells that are clonally derived from an original tumourigenesis in a long dead host and are genetically distinct from their current host individual. Thus, tumours can be regarded as a highly degenerate obligate parasitic mammal. Transmissible tumours share most of the characteristics of a typical microparasite, such as prolific replication within the host (McCallum & Jones, in press). Transmission of this cancerous cell line is possible due to low host genetic diversity, particularly in the Major Histocompatibility Complex (MHC) (Siddle *et al*. 2007), a complex of genes responsible for generating immune responses in vertebrates. Thus, the immune system of the devil fails to recognise foreign tumour cells as non-self, and the tumour is passed on as an allograft between susceptible and infected hosts (Pearse & Swift, 2006). To date, DFTD has been proved to be consistently fatal, with infected individuals succumbing to the disease between six to twelve months after the detection of clinical signs.

DFTD has spread from the point of its first detection in northeastern Tasmania to now occupy most of the devil's distribution. The west and north western areas of Tasmania now contain the only remaining DFTD-free populations (McCallum *et al.* 2007, 2009). The typical pattern following DFTD arrival is a rapid increase in disease prevalence to more than 50% and severe declines in population size and survival rates within four years (Lachish, Jones & McCallum 2007; McCallum *et al.* 2009). Our recent study in a

northwestern Tasmanian devil population at "West Pencil Pine", with an MHC composition different from both the tumour and eastern populations (Siddle *et al.* 2010) demonstrated, for the first time, reduced impacts at the population and individual level (Hamede *et al.* 2012). Reduced impacts at West Pencil Pine were characterized by much slower decline in population growth rates and population size, no change in age structure, increased longevity of infected individuals and slower increase in prevalence of DFTD, compared to three eastern populations affected by DFTD (Hamede *et al.* 2012). Three different mechanisms could explain these patterns at West Pencil Pine compared with other eastern sites. First, differences in genetic structure of the host populations could manifest as either resistance or tolerance to DFTD (see Ebert & Bull 2003; Carval & Ferriere 2010), leading to reduced prevalence or slower disease progression, respectively. MHC genotypes, which are involved in tumour recognition, are different at West Pencil Pine from those of eastern populations (Siddle *et al.* 2010). Second, the patterns could result from a less virulent strain of the tumour, a hypothesis supported by evidence of a tetraploid DFTD strain being present in the West Pencil Pine population (A.M. Pearse pers. comm.). Third and of relevance to this paper, reduced agonistic behaviour resulting in lower biting injury rates could explain the reduced infection risk at West Pencil Pine (Hamede *et al.* 2012).

Acquiring knowledge of transmission in wild animals is notoriously difficult. The clonal nature of the devil facial tumours is evidence for direct transmission of tumour cells from infected to susceptible host individuals (Pearse & Swift 2006; Siddle *et al.* 2007). One of the requirements for the evolution of a transmissible cancer is intimate injurious contact that brings live tumour cells in direct contact with sub-epidermal tissue of the susceptible host in a suitable location where they can grow (McCallum & Jones,

in press). Transmission must be swift as live cells do not survive long outside the body. This points towards biting as the major route of transmission, although transmission through fomites have not been excluded. Tasmanian devils are predatory carnivores with substantial canine teeth (Jones & Stoddart 1998; Jones 2003). They bite each other and inflict wounds that range from canine tooth puncture holes to sizeable avulsions (Hamede, McCallum & Jones 2008).

Several lines of evidence indicate that transmission of DFTD is strongly frequency dependent. The force of infection remains high, at around 50%, even when populations are severely depleted by the disease to very low densities (McCallum *et al.* 2009). In addition, the disease is spreading into naturally very low density populations (McCallum *et al.* 2007). This suggests frequency dependent transmission, which is typical of sexually transmitted diseases, and which has no threshold population density below which the disease will die out (McCallum, Barlow & Hone 2001). DFTD transmission shares some attributes with sexually transmitted diseases. Most of the biting injuries occur during the mating season (Hamede, McCallum & Jones 2008), due to male contests and females evading mate guarding (M. J., unpublished data), with just a low background level of bites the remainder of the year that would occur during feeding and other social interactions (Hamede, McCallum & Jones 2008). Tasmanian devils are specialized scavengers and social interactions around carcasses involving biting behaviour are common (Pemberton & Renouf 1993; Hamede, McCallum & Jones 2008). While the rate of close proximity contacts during feeding interactions increases with population density (Hamede, McCallum & Jones 2008), few of these feeding encounters result in injury (Pemberton & Renouf 1993).

Our aim in this study is to investigate whether the frequency of biting injuries predicts future acquisition of DFTD in individuals. We relate the seasonal patterns of the number and location of bites in individuals to subsequent development of tumours in those individuals in two populations that subsequently differed in epidemic outcomes; disease prevalence and population effects of DFTD. We discuss our results in relation to devil behaviour and ecology that is relevant to DFTD transmission.

Materials and methods

Study sites and data collection

We analysed data from two different DFTD affected populations in northern Tasmania (West Pencil Pine and Wisedale, Fig. 1) that were sampled using identical protocols. We set 40 custom built devil traps (constructed of 300mm polypipe) baited with meat and checked commencing early each morning, over a 25km^2 area for 10 nights, four times a year at three month intervals. The timing was chosen to sample from four important seasons that represent key life-history events: summer (February, when juveniles are dispersing prior to the mating season), autumn (May, immediately after the mating season), winter (August, when females are carrying pouch young) and spring (November, when females are in late lactation). West Pencil Pine (41^o 31' S, 145^o 46' E), is a 25km^2 area situated on private production forestry land to the west of Cradle Mountain in northwest Tasmania. This population was monitored from August 2006 (in the early stages of disease arrival at the site) until May 2010. Wisedale (41^o 16' S, 146^o 39' E), is a 25km^2 area situated on a private farm in northern Tasmania which was monitored from May 2006 (in the early stages of disease arrival at the site) until February 2008. To determine the pattern of injuries between individuals in both populations, we recorded the presence and location of all injuries that resulted in

penetration of the dermal layer and therefore might have the potential to transmit DFTD. Bites resulting in this type of injury usually heal within two to eight weeks depending on their severity (R.H. and M.J., personal observation), thus the three month intervals at which data was collected precluded double counting bites in subsequent trapping sessions. In addition, only unhealed bites, those bites that presented visible disruption of the epidermis, were included in the data analysis. Injuries resulting from agonistic interactions with other carnivores are extremely rare (M.J., unpublished data).

All devils were individually marked with microchip transponders (Allflex New Zealand). Disease status was assessed by histopathological examination of biopsies from tumours, or when this was not possible, by visual inspection and identification of tumours (see Hawkins *et al.* 2006 for visual detection methods). In our data set we have only categorized individuals as DFTD infected if confirmed by histopathology analyses or if visually scored as definitive DFTD (Hawkins *et al.* 2006). We recorded the location (inside the oral cavity or outside) of primary tumours (which are invariably on the head) in all individuals. When captured individuals had more than one tumour, the larger tumour was regarded as the primary tumour.

Devils were aged using a combination of molar eruption, molar tooth wear and canine over eruption (distance from dentine-enamel junction to the gum) (M.J., unpublished). This method is considered precise for ageing devils up to 3 years of age. Because both sites were monitored regularly, most individuals in our data set were captured as juveniles or younger than 3 years old and were therefore of known age. In our analyses we excluded subadult devils (1 year old) as they usually do not get bitten until they become sexually mature (2 years old).

Statistical analysis

All analyses were implemented in R version 2.12 (R Development Core Team, 2011). At West Pencil Pine, to determine whether the number of bites on an individual influenced its probability of acquiring DFTD in the future, we used Generalized Mixed Models, implemented using the lme4 package in R (Bates, Maechler & Dai 2008) with individual devils as a random term (to allow for the fact that individual devils were repeatedly captured) and a binomial error distribution. All models were fitted using maximum likelihood, which is appropriate when examining fixed effects (Crawley 2007). For all cases in which devils had been captured and then recaptured either six or nine months subsequently, we examined whether the DFTD status (healthy or diseased) was associated with the number of bites recorded six or nine months previously. We chose six and nine month intervals as these time periods represent the best estimates available for the latent period of DFTD based on field (R. Hamede, unpublished data) and experimental observations (Kreiss *et al.* 2010). Because almost all primary tumours occur on the head (R.H. and M.J. personal observation) we ran separate analyses using bites to the head only, bites to the body tail and limbs, and all bites pooled. We restricted this analysis to West Pencil Pine as this was the only site at which individual devils were consistently recaptured throughout the sampling period.

Seasonal patterns of bites in both sites were analysed using Generalized Linear Mixed Models (GLMM) with normal error distribution, using the package lme4 (Bates, Maechler & Dai 2008). There was substantial variation in the mean number of bites per season between years at each site. We therefore used GLMM to test the fixed effects of season and site, with the interaction of year, site and season as the random term. As we

were again comparing fixed effects, the models were fitted using maximum likelihood rather than restricted maximum likelihood.

We obtained population size estimates from mark-recapture data using MARK (Cooch & White 2002). Closed population estimates were obtained including heterogeneity in capture probabilities with time and between individuals (Chao, Lee & Jeng 1992), implemented in the program CAPTURE (Rextad & Burnham 1992). Population size estimates and 95% confidence intervals were converted to population density by dividing population size by the combined area of a minimum convex polygon constructed around the trapping grid (Kenward 1985) and an additional two kilometre boundary strip that represents half the home range diameter of an individual devil (Pemberton 1990).

Results

At West Pencil Pine, adult Tasmanian Devils with a large number of total bites or bites elsewhere than the head were significantly less likely to develop DFTD after either a six month delay or a nine month delay (Table 1) than devils with few bites. Devils with less than 5 total bites were more than twice as likely to develop DFTD as those with between 5-9 bites (Fig. 2 (a) and (c)). In contrast, we found no significant (P=0.05) evidence that the number of bites to the head affected the likelihood of developing DFTD in either six months time or nine months time (Table 1), despite the majority (55%) of all bites recorded being to the head. There was no significant evidence that the probability of developing a tumour was influenced either by sex or age and the effect of total bites on the likelihood of developing DFTD remained significant when either age or sex was included in the model (Table S1). Primary tumours were predominantly

observed inside rather than outside of the oral cavity at both sites (Fig. 3). There were no subadults infected with DFTD at West Pencil Pine.

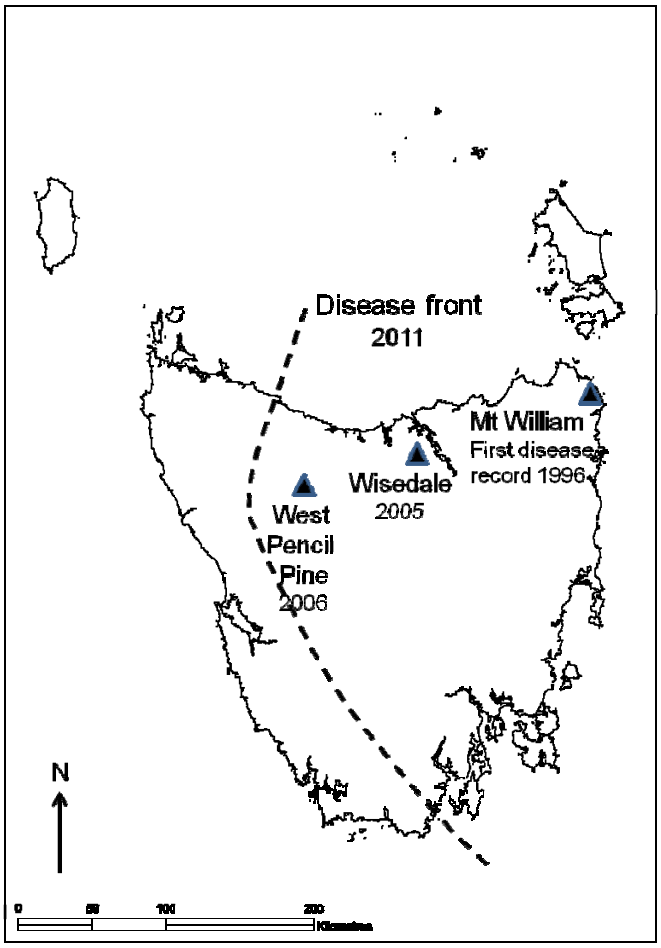

Figure 5.1 A map of Tasmania showing the location of the two study sites, the current disease front and the location of the first record of Tasmanian devil facial tumour disease.

Time delay	Model	df	AIC	Log-Likelihood	Chisq	df	Pr(>Chisq)	Coefficient	SE
6 months	Intercept only	2	192.76	-94.382					
	All bites	3	186.79	-90.393	7.97	1	0.0047	-0.212	0.081
	Bites to head	3	193.97	-93.985	0.79	1	0.37	-0.091	0.122
	Non-head bites	3	181.13	-87.571	13.62	1	0.0002	-0.601	0.221
9 months	Intercept only	2	183.17	-89.58					
	All bites	3	176.29	-85.14	8.87	1	0.003	-0.253	0.108
	Bites to head	3	184.25	-89.12	0.91	1	0.34	-0.103	0.132
	Non-head bites	3	166.64	-80.92	18.56	1	0.00001	-0.993	0.415

Table 5.1 Results of generalised models predicting the probability of developing DFTD as a function of the number of bites recorded six months or nine months previously, for all bites, bites to the head only and "non-head bites" (to the body, tail or limbs). Chi-squared tests for likelihood ratios relative to a null model containing only the intercept are shown, together with the estimated coefficient and standard error for the effect of the number of bites on the logit transformed probability of acquiring DFTD. In these analyses, the actual number of bites recorded was used as a predictor variable (i.e. the bites were not aggregated into categories as in figure 2). Analyses based on 600 observations from 107 Tasmanian Devils.

Model	lower	upper
(Intercept)	4.371974	6.403533
season spring	-2.72649	-0.12218
season summer	-3.37345	-0.59375
season winter	-1.53622	1.354017
site wisedale	-0.27276	2.088572

Table 5.2 Highest posterior density estimates (95% confidence) of parameters for a generalized mixed model with year:site:season as the random term, predicting the total number of bites as a function of season and site, with season parameters relative to autumn and the site parameter relative to West Pencil Pine. MCMC sample with n=1000. based on 748 individual capture events from West Pencil Pine and 116 from Wisedale.

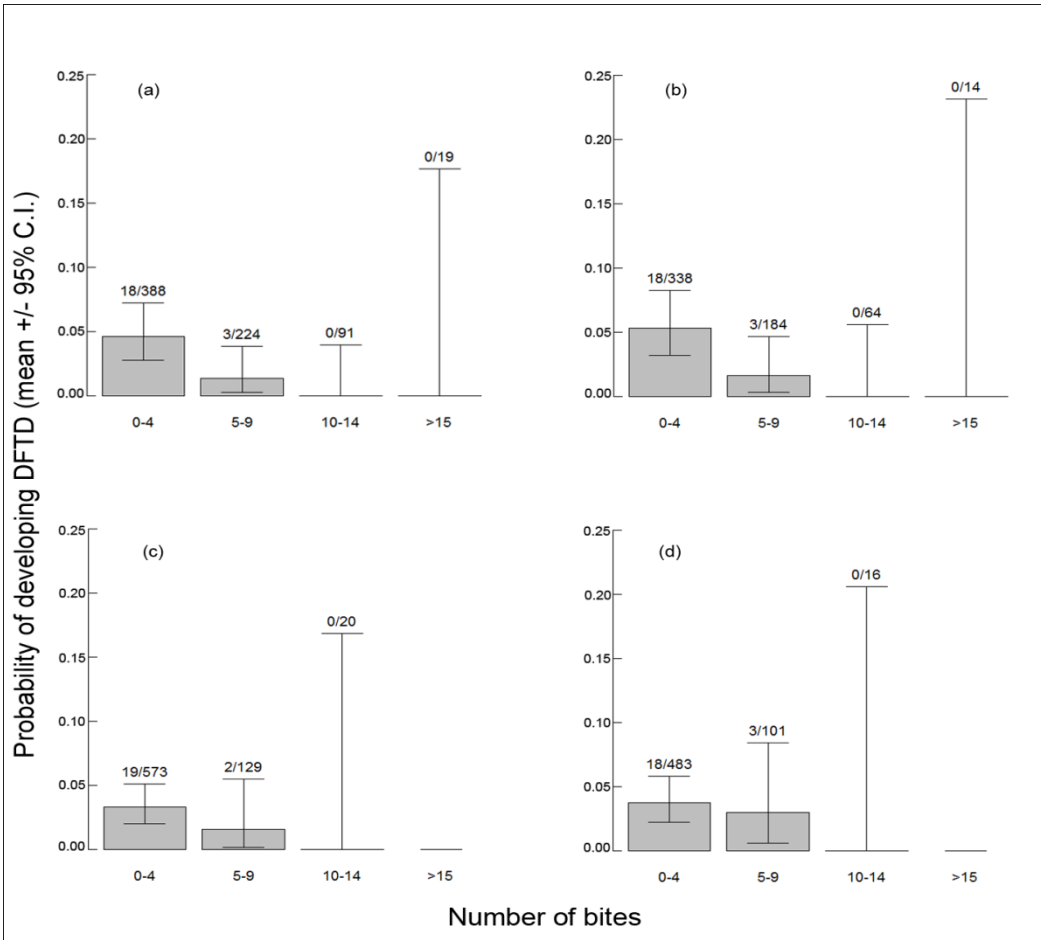

Figure 5.2 The probability of a Tasmanian devil developing DFTD in the future as a function of the number of bites recorded on that animal. (a) DFTD 6 months in the future, total bites; (b) DFTD 9 months in the future, total bites; (c) DFTD 6 months in the future, bites to the head; (d) DFTD 9 months in the future, bites to the head. The error bars are exact binomial 95% confidence intervals. The number of DFTD cases as a fraction of the number of observations contributing to the estimate is shown above each bar.

Bites in subadults were very rare at both sites regardless of season (Table S2).

The number of bites in adult devils was greater at both sites in autumn and winter than in spring and summer, although there was substantial inter-annual variation, particularly at West Pencil Pine (Fig. 4). Generalized mixed modeling using the interaction between site, year and season as the random term showed that a model including a seasonal effect but no site effect was most strongly supported by the data, with the number of bites being lower in spring and summer than in autumn (Table 2). Estimated population density was consistently lower at Wisedale than at WPP, in all three years for which estimates were available at both sites (Fig. 5).

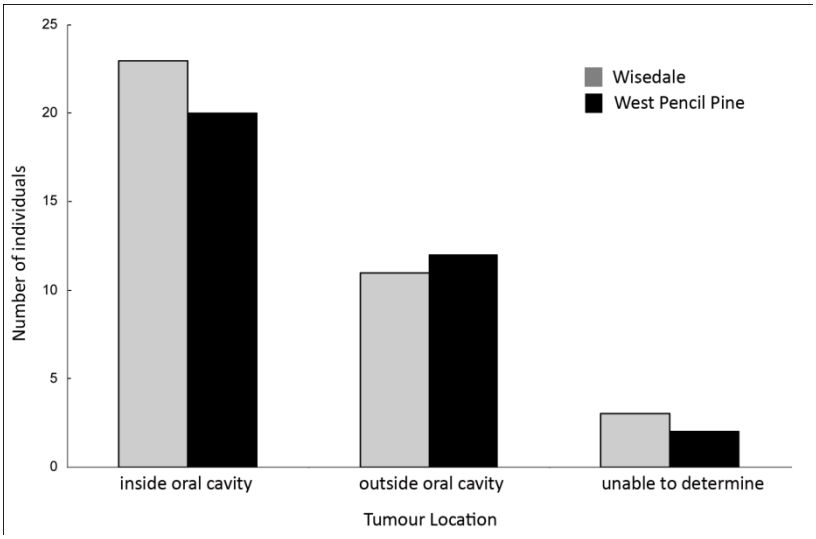

Figure 5.3 The location of primary tumours in diseased individuals at West Pencil Pine (black) and Wisedale (grey).

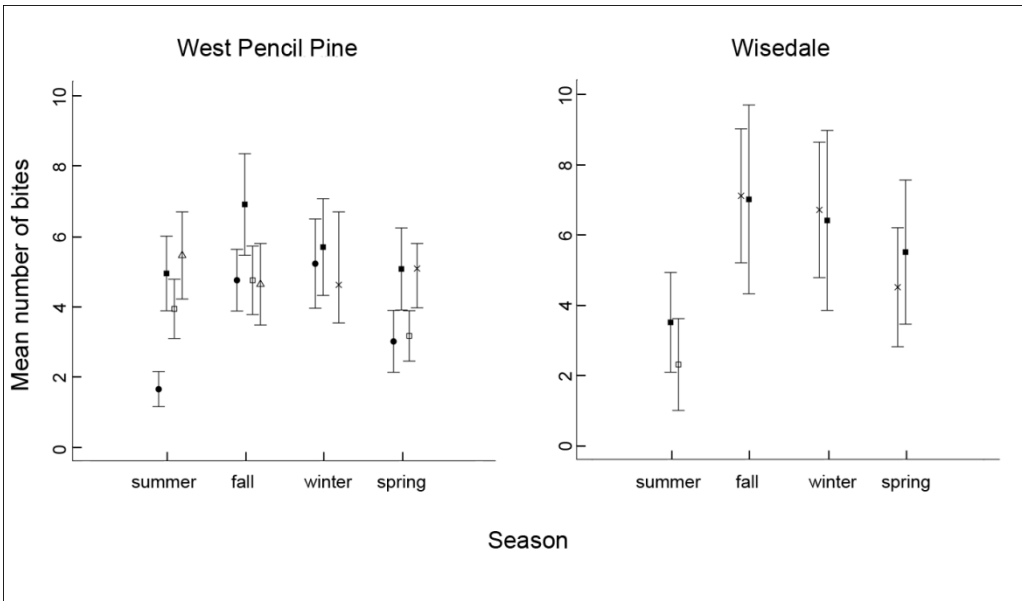

Figure 5.4 Seasonal patterns in the mean number of bites at West Pencil Pine and Wisedale in different years (cross = 2006; solid square = 2007; open square = 2008; solid circle = 2009 and open triangle = 2010).

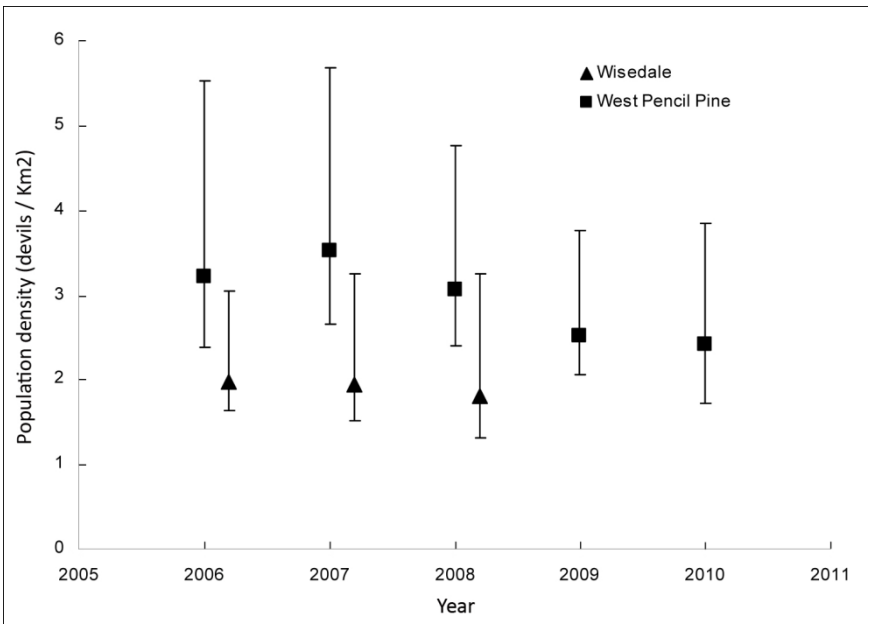

Figure 5.5 Estimates of population density (devils per km^2) with 95% confidence intervals at West Pencil Pine (squares) and Wisedale (triangles). The areas of the sites (including the 2 km boundary strip) are 81 km^2.

Discussion

Whilst there is very strong evidence that DFTD is transmitted through transfer of live tumour cells between hosts (Pearse & Swift 2006), how transmission occurs in the field has remained unclear. Transmission has been presumed to occur through biting (Pearse & Swift 2006; Siddle *et al.* 2007). Transfer through fomites has not been discounted but is unlikely given that tumour cells do not survive for more than a few minutes outside the body of a devil (Tasmanian Department of Primary Industries, Parks, Water and Environment [DPIPWE], unpublished data). There are two ways in which transmission through biting could occur: from the biter to the bitten, or from the bitten animal to the biter. If transmission occurred predominantly by the former route, one would expect that those animals with more bites, particularly to the head (where primary tumours occur) would be those that should subsequently have a higher probability of developing DFTD. Tumour cells and clusters of cells have been swabbed from canine teeth of infected devils with ulcerated tumours in close proximity to the tooth. Transmission potential is assumed to increase with tumour size, as ulceration (Loh et al. 2006) and thus friability increases with tumour size and age. No tumour cells were found in canine smears from devils that had non-ulcerated tumours or tumours outside of the oral cavity (Obendorf & McGlashan 2008).

Our results are more consistent with transmission occurring predominantly by a susceptible animal with injuries and exposed flesh inside or around the mouth biting into the tumour of an infected individual, a behaviour we have observed in wild devils (R.H. and M.J., personal observation), than through transfer of cells from biter to bitten. We found that devils with fewer bites overall, or elsewhere than the head, were

significantly more likely to have developed tumours after either six or nine months (Fig. 2, Table 1), and that this was not related to either age or sex (Table S1). We did not find any evidence of a relationship between propensity to develop DFTD and the number of bites to the head only. Our unexpected result that devils with fewer bites overall or elsewhere than the head are more likely to acquire DFTD is explicable if the more aggressive animals, which bite more frequently than more subordinate individuals, are themselves less likely to be bitten, particularly on locations other than their head. The lack of a relationship between propensity to acquire DFTD and bites to the head is explicable if aggressive animals attacking others head-on tend themselves to get bitten on the head.

The location of the majority of primary tumours inside the oral cavity (Fig. 3) adds additional support to biting infected devils as the behaviour most likely to lead to infection with DFTD. If tumours were largely transferred through an infected individual biting a susceptible individual, we would expect tumours to be predominantly external. Whilst we do not have unequivocal evidence that tumours occur close to the site of original inoculation, in the small number of infections that have been experimentally induced by injecting or implanting tumour cells, the primary tumour has occurred at the site of the original inoculation (S. Pyecroft pers. comm., G. Woods, pers. comm.). As specialised scavengers that eat the tough parts of large prey carcasses (Jones 1997), devils frequently have open wounds on their lips or inside their mouth, either from biting each other or from feeding on bones or sharp food items such as echidna (*Tachyglossus aculeatus*) spines (R.H. and M.J, personal observation). As a devil bites into the tumour of another devil, tumour cells could easily be embedded in such open

wounds, particularly if the tumour was friable or ulcerated, leading to the growth of a tumour at the site of injury inside the mouth.

Patterns of biting and of social contacts in wild Tasmanian devils show clear seasonal differences (this study, Hamede, McCallum & Jones 2008; Hamede *et al.* 2009), even with substantial inter-annual variation, although this does not translate into a seasonal distribution in new cases of DFTD (McCallum *et al.* 2009). Biting rates at West Pencil Pine, at Wisedale and at a third site, the Freycinet Peninsula, on the East Coast of Tasmania (Hamede, McCallum & Jones 2008) were higher in autumn and winter following the mating season when male-male contests and intersexual interactions (mate choice, mating and mate guarding) peak. These results are concordant with different patterns of social contact, as revealed by proximity sensing radiocollars, between mating and non-mating seasons (Hamede *et al.* 2009). A distributed delay in the latent period of the disease in the wild, due to the number and location of cells transferred, natural variability in host susceptibility (genotype) and immunological response, or overall individual fitness may explain the lack of a seasonal trend in prevalence of DFTD (McCallum *et al.* 2009) despite seasonality in contacts and biting injuries. The latent period from experimental inoculations of pieces of tumour and tumour cells from cultured cell lines range from one to four months (Kreiss *et al.* 2010). However, such inoculations probably transfer more cells directly into a potential site for tumour establishment than would be the case for field transmission, and thus are likely to have a shorter latent period than in the wild. Data on latent period from wild populations comes from a single anecdotal case in which a wild devil brought into captivity developed DFTD ten months after its removal from the wild (DPIPWE, unpublished data).

We found no evidence of a significant difference in the mean number of bites between sites, despite the higher population density at West Pencil Pine than at Wisedale (Fig. 5), suggesting that the reduced impact and low progression of DFTD reported at West Pencil Pine (Hamede *et al.* 2012) is unlikely to be due to reduced contact rates. Two separate observations support why this might be the case. First, while the frequency of contacts observed at carcasses placed at feeding stations increased with population density (Hamede, McCallum & Jones 2008), when devils have been subsequently trapped and assessed for injury following behavioural observations (not done in Hamede, McCallum & Jones 2008), agonistic interactions are found to rarely result in penetrating injuries (Pemberton & Renouf 1993). Second, population density probably does not play a major role in the transmission dynamics of DFTD. Epidemiological (McCallum *et al.* 2009) and social network studies (Hamede *et al.* 2009), have suggested that transmission of DFTD is more consistent with a frequency-dependent than a density-dependent mode. This is consistent with the majority of biting occurring during mating encounters rather than during foraging interactions (Hamede, McCallum & Jones 2008).

Options for managing the disease and mitigating its impact on wild populations are limited. In the absence of any treatment for DFTD or a vaccine, nor prospects for either in the foreseeable future, and with the difficulties of controlling DFTD by selective culling (Lachish *et al.* 2010), identifying and manipulating host or tumour traits that are under selective pressure are possible management actions that could reduce transmission. Individual level genotypic and phenotypic variation in aggression is common in animal populations (Sih, Bell & Johnson 2004; van Oers *et al.* 2005).

Identifying, with the view to systematically removing, highly aggressive phenotypes or genotypes that have the potential to be super spreaders could be a viable management for DFTD affected populations. This type of management could enhance existing natural disease-induced directional selection on behavioural traits, and lead to the rapid evolution of a less aggressive devil species that is more resilient to DFTD. If more aggressive individuals are more likely to become infected, and these individuals die in less than 12 months since infection or are selectively removed in management actions, individual fitness outcomes should drive selection for lower aggression.

Determining behavioural and ecological circumstances associated with high risk of disease transmission is a critical step for understanding the epidemiology and impact of pathogens and parasites in their hosts (Hart 1988; Courchamp *et al.* 1998). Our results highlight the importance of investigating parasite and pathogen transmission in the wild in order to understand the dynamics of infectious diseases of wildlife and emphasise the role that heterogeneity in individual behaviour may play in susceptibility to infectious disease.

Supplementary Information

Table S5.1 Likelihood ratio tests for generalised mixed models predicting the probability of developing DFTD as a function of the number of bites recorded either six months or nine months previously and the factors, sex or age. In each row, the chi-squared statistic for +factor is the likelihood ratio when the factor is added to a model including the number of bites and the chi-squared statistic for +bites is the likelihood ratio when the number of bites is added to a model including the factor.

Factor	Bite location	Delay (months)	Chisq+Factor	p	Chisq+Bites	p
sex	head	6	1.52	0.21	0.38	0.53
sex	head	9	1.21	0.27	0.52	0.46
sex	total	6	0.36	0.54	6.41	0.011
sex	total	9	0.161	0.68	7.43	0.006
age	head	6	0.407	0.52	0.88	0.34
age	head	9	0	0.98	0.91	0.34
age	total	6	0.44	0.51	8.08	0.004
age	total	9	0.09	0.75	8.97	0.003

Table S5.2 Mean number of bites and standard error in subadult devils at West Pencil Pine and Wisedale.

Site	Year	Season	Bites	SE
West Pencil Pine	2007	summer	0	0
West Pencil Pine	2008	summer	0.54	0.12
West Pencil Pine	2009	summer	0.15	0.07
West Pencil Pine	2010	summer	1.21	0.29
West Pencil Pine	2007	autumn	0.07	0.05
West Pencil Pine	2008	autumn	0	0
West Pencil Pine	2009	autumn	0.5	0.17
West Pencil Pine	2010	autumn	0.52	0.22
West Pencil Pine	2006	winter	0.43	0.15
West Pencil Pine	2007	winter	0.77	0.16
West Pencil Pine	2009	winter	1.42	0.29
West Pencil Pine	2006	spring	1	0.4
West Pencil Pine	2007	spring	0.77	0.16
West Pencil Pine	2008	spring	0.54	0.15
West Pencil Pine	2009	spring	1.1	0.34
Wisedale	2007	summer	0.26	0.11
Wisedale	2008	summer	0.88	0.3
Wisedale	2006	fall	0.25	0.11
Wisedale	2007	fall	0.58	0.2
Wisedale	2006	winter	2.07	0.52
Wisedale	2007	winter	1.09	0.26
Wisedale	2006	spring	1	0.5
Wisedale	2007	spring	1.24	0.27

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CHAPTER 6

General Discussion

Research Summary

This thesis provides an integrated and multidisciplinary approach to develop an understanding of the transmission dynamics and ecology of DFTD in wild Tasmanian devil populations. To achieve this, I examined three major aspects of the host-pathogen system which are directly relevant for disease transmission. First, this study provides a thorough assessment of host contact heterogeneities within a social network analysis framework that includes demographic and seasonal variation. In addition, I use this information to build disease simulation models to test basic assumptions of traditional compartmental models and to predict the transmission dynamics, epidemic outcome and impact of DFTD. Second, I estimate the dynamics of behaviours directly associated with DFTD transmission (biting and contact rates) and its relationship with ecological, seasonal and life history events of the host. Finally, I use longitudinal epidemiological data sets from two genetically distinctive provenances to compare and follow the transmission dynamics, progression and impact of DFTD in wild populations.

The results of this study have direct implications for improving the management of this emerging and extinction threatening disease and provide new insights and avenues for the conservation of Tasmanian devils in the wild. The effect of disease in wildlife populations can act in synergy with other major endangering processes such as habitat loss, loss of genetic diversity or introduction of pest species. Thus, understanding the role that pathogens play in population dynamics and their overall effect on host populations, host evolution and genetic diversity is a complex task. Conservation managers are often ill-equipped to reduce the risk of disease to wildlife and to mitigate its impact for several reasons. First, understanding disease dynamics

and assessing their impact on wildlife populations requires robust long-term data sets, which not always are logistically or economically feasible to undertake, especially for endangered wildlife. Second, tools for responding to disease outbreaks are often limited by a number of factors such as the ability to thoroughly understand the transmission dynamics of the disease, the mechanisms involved in disease transmission and the ecology of the host-pathogen system. Finally, emerging infectious disease affecting naïve populations are under strong selective and evolutionary pressure (Altizer et al. 2003; Ebert and Bull 2003), thus, predicting trade-offs and adaptive responses in the host-pathogen system is a complex but essential task.

Here, I discuss the extent to which our understanding of the transmission dynamics of DFTD has been advanced by this study and integrate the results from each chapter to identity key areas for future research and to outline potential disease management strategies. I interpret the transmission dynamics and impacts of this novel infectious disease with a multidisciplinary perspective, using the most recent advances in DFTD diagnostic, cytology and immuno-genetic research. The conclusions drawn from this study are broadly applicable to a range of other conservation challenges posed by wildlife diseases.

The transmission dynamics and behavioural ecology of DFTD and its relevance for understanding the ecology and epidemiology of wildlife infectious diseases.

Social behaviour and pairwise interactions between individuals play a central role in the ecology of wildlife populations and in the transmission of infectious diseases. This study reveals that the dynamic contact networks of Tasmanian devils have direct implications for the transmission and spread of DFTD. Describing the structure of a contact network and its seasonal dynamics also provides a rational basis for developing more effective strategies for understanding and managing epidemic outbreaks in wildlife (Proulx et al. 2005). In addition, my results add important new information about devil ecology and social behaviour. Until now, contact rates and devil behaviour were restricted to observations around food sources (Jones 1998; Pemberton and Renouf 2003; Hamede et al. 2008) and understanding of devil behaviour during the mating season was limited.

Seasonal variation is arguably one of the most ubiquitous sources of variation in wild populations and has been found to be a particularly relevant factor driving contact rates and dynamics of infectious diseases (Begon et al. 1999; Hosseini et al. 2004; Altizer et al. 2006). Moreover, seasonal changes with high physiological demands such as molting or migrating can have broader implications for disease transmission, affecting host susceptibility and immune dynamics within the host-pathogen system (Norris and Evans 2000). The seasonal effects on immune responses are best documented in migratory birds. Migrating birds have shown higher levels of

immunosuppression than conspecifics outside of the migratory season, which has direct implications for the dynamics of infection (Owen and Moore 2006).

Determining the extent to which important seasonal events such as reproduction can alter host immune responses or mediate resistance and tolerance to pathogens is an important step in understanding transmission dynamics in wild populations. An example that highlights the importance of breeding season changes in host behaviour and costly immune defences in driving transmission dynamics is the interaction between the European rabbit and a nematode parasite. Nematode (*Trichostrongylus retortaeformis*) infestation was greater during spring and summer in breeding adult female rabbits than in adult males and non-pregnant females as a result of periparturient rise (Cattadori et al. 2005), a process in which females exhibit low immunity to prevent harming their fetuses (Loyd 1983). An important challenge, however, is determining the trade-offs involved in balancing these immunological costs between host reproduction and pathogen infection and the extent to which they can mediate selective processes such as disease tolerance or resistance. This study indicates that the mating season represents a high risk period for transmission of DFTD via an increase in contact frequency, contact length, and bite injury frequency in wild Tasmanian devils. The data also reveal that sexually mature males share dens for extended periods with several females during the mating season (Jones et al. 2007), thus providing further opportunities for increased contact rates and disease transmission. Furthermore, seasonal immunosuppression during breeding, triggered by sustained levels of corticosteroids, which is well documented in semelparous carnivorous marsupials (Oakwood et al. 2001; Schmidt et al. 2006), and likely in Tasmanian devils which have elevated levels of stress hormones during the mating

season (Hesterman 2008; Hesterman and Jones 2009), could further increase disease susceptibility. The extent to which these seasonal physiological costs can impact the devil's immune responses and disease tolerance is, however, unknown and merits further investigation within an ecological context. Knowledge of the times of the year or other specific conditions under which host immune responses can be lowest can also improve targeted management of at-risk populations or demographic groups.

The social behaviour of individuals has direct implications for within and between host processes involved in disease transmission. While host behaviour can alter the likelihood of acquiring infection at the individual level, it can also affect the transmission rate between individuals and infection dynamics at the population level. Therefore animal behaviour represents a critical area of investigation for integrating disease ecology and epidemiology (Hawley and Altizer 2011). DFTD is an infectious disease transmitted by biting, therefore information on host aggressive behaviour and bite injury intensity and its interaction with ecological factors can be particularly useful for understanding disease transmission in the wild (Totton et al. 2002; Delahay et al. 2006). My study reveals that devils with fewer injuries are more likely to develop DFTD than those with more bites, and that primary tumours occur mostly inside the oral cavity. These counterintuitive results suggest that more aggressive individuals (those that deliver bites more frequently and receive bites less often) could be at higher risk of developing DFTD by biting into the tumour of infected individuals. While population density is positively correlated with contact rate at carcasses (Hamede et al. 2008), it seems to have no effect on bite injury rates. The possible effect that aggressive phenotypes could have in the transmission dynamics of DFTD merits further research.

Parasites and pathogens can change host behaviour in diverse ways, affecting foraging patterns, anti-predator responses, mating and agonistic behaviour, parental care and investment in sexual reproduction, all of which directly impact their fitness and potential for pathogen transmission (Hart 1988; Moore 2002; Wendland et al. 2010). Behavioural changes as a result of infection are termed 'sickness behaviour' and can reduce immune responses (Hart 1988; Adelman and Martin 2009). In male house finches, for example, infection with avian mycoplasmosis (*Mycoplasma gallisepticum*) resulted in reduced aggressive behaviour which attracted healthy conspecifics seeking to avoid behavioural aggression at feeding stations (Bouwman and Hawley 2010). The effect of DFTD on the agonistic behaviour of Tasmanian devils is unknown and deserves attention. Incorporating pathogen induced behavioural changes into epidemiological models can also help in determining the extent to which infection status can alter contact rates between hosts and its broader implications for disease dynamics (Funk et al. 2009)

Understanding the trade-offs involved in sickness behaviour in wildlife diseases within an ecological and adaptive framework can help to identify the key processes that affect the persistence of infection and the coexistence of host and pathogens. These trade-offs could be particularly relevant for the devil-DFTD system. DFTD is an infectious cancer with 100% mortality occurring in less than one year following clinical signs and Tasmanian devils have a short life span (usually 5-6 years in the wild). Therefore, behaviours that increase the fitness of infected devils would be under strong selective pressure. Because sickness behaviours should be adaptive (Hart 1988; Adelman and Martin 2009), these trade-offs could also shape host life history,

social and mating systems in wildlife populations (Hart 1988; Martin et al. 2008; Wendland et al. 2010). This point emphasizes the importance of including animal behaviour and the effect of disease on host behaviour in model parameterisation to better characterize host-pathogen dynamics and evolutionary processes operating in disease ecology.

To accurately capture the relationship between disease dynamics and contact patterns, it is crucial to go beyond the random mixing assumption of transmission in epidemiological models. Network studies have provided two novel and important improvements in comparison to traditional compartmental disease models: data able to reproduce the real complexities and heterogeneities of contact between individuals and a quantitative and qualitative framework from which to test hypotheses about network function in epidemic behaviour. My study is one of the few that uses comprehensive empirical data from a novel technology, proximity-sensing radio collars, able to accurately estimate the frequency and nature of interactions in wild animals. This approach enables the identification of key subgroups or individuals that have the potential to be qualitatively and quantitatively more likely to transmit disease. Although the population I studied was not affected by DFTD at the time and the progress of the epidemic was not followed after disease arrival, (due to ethical concerns regarding attaching radio-collars to diseased individuals), important lessons can be taken from the network approach. Significant differences in devil interactions are characterized by seasonal changes in global connectivity and individual variability in frequency and length of contact rates related to the mating season. These results suggest that DFTD is effectively behaving like a sexually transmitted disease, which has frequency dependent transmission.

My study has refined the parameterization of two key epidemiological aspects in modeling disease spread in contact networks which have direct relevance for better understanding the transmission and ecology of wildlife diseases: estimating individual-based heterogeneities in mixing patterns and incorporating temporal dynamics of network structure. I use tuneable algorithms and formulas to test model assumptions about contact patterns and the impact of population structure on disease transmission through dynamic contact networks. This offers a computationally tractable framework to predict and model epidemic outbreaks in wildlife diseases. Most network models incorporate social contacts with the underlying assumption that mixing rates are static, which means that once an association has been formed between individuals or groups they will remain unaltered. Nonetheless, because of the great diversity in individual behaviour and social structure in natural populations this assumption is rarely a realistic approximation of the real contact patterns in social networks and usually fails to yield reliable estimates of disease dynamics (Fefferman and Ng 2007). Furthermore, network models usually generalize the dynamic processes of infection and maintain important global network metrics such as mean degree and clustering fixed in time. My study uses dynamic contacts networks by shifting associations between individuals according to key ecological, seasonal and demographic characteristics of the host. By doing so, it is possible to assess the role of both local and global properties of contact networks in disease dynamics.

Network metrics play an important role in the spread of infectious diseases and provide critical information to understand the relationship between host social structure and dynamics of infection within and between populations. An important

structural aspect of the devil social network is that a few individuals in the collared population have unusually high values of two important metrics in terms of their relation to other nodes in the network: degree and betweenness centrality. Degree centrality is a measure of the number of contacts an individual has in the network whereas betweenness centrality (also called *shortest path betweenness*) is the extent to which an individual serves as a "go between" for other pairs in the network (Freeman 1977). Both metrics have been found to have critical relevance for disease transmission in many host-pathogen systems (Corner et al. 2003; Christley et al. 2005). Network studies able to capture the complexities of contact patterns can also provide valuable insights into the relationship between host social structure and epidemiology. For example, the epidemic dynamics in highly social and territorial species such as meerkats (*Suricata suricatta*) and African lions (*Panthera leo*) are characterized by contact patterns with high clustering and betweenness (Drewe 2010; Craft et al 2010), which are characteristic of small-world networks. While clustering produces a strong saturation effect by keeping the pathogen locally restricted but well connected, the short path lengths associated with high betweenness allow transmission between different social groups (Craft et al. 2010; Drewe et al. 2010). Although Tasmanian devils are neither territorial nor group-living the structure of our empirically estimated contact network resembles, to a certain extent, those of social and territorial species. The observed devil networks both inside and outside of the mating season had significantly higher levels of clustering than expected in random networks. In addition, only a few individuals had considerably high values of betweenness and degree in both seasons. This might explain why DFTD has not disappeared from any local population after its arrival. While high global connectivity and betweenness ensures the pathogen can easily reach most of the population,

individuals with high degree can decrease the epidemic threshold of the disease. In addition, the frequency dependent mode of transmission in DFTD means that there is no population size threshold for the disease to die out.

Identifying if host sex has a significant role in disease dynamics has also been a priority for epidemiological research (Poulin 1996; Sheridan et al. 2000; Skorping and Jensen 2004). For example, the transmission of tick-borne encephalitis in the yellow-neck mouse (*Apodemus flavicollis*) has been shown to be mainly driven by males (Perkins et al. 2003; Ferrari et al. 2004). If disease dynamics are driven by one sex, then targeting control and management strategies in that sex should be, in principle, more effective and practical to deliver. Another important finding from this study is that although the associations between females were both more frequent and longer than between males, values for individual-based network metrics did not significantly differ between sexes. In fact, male-female associations accounted for more than 95% of all the associations based on the frequency and length of interactions during the mating season. As this is the season when contact frequency and length increases it is unlikely that one particular sex could have a more relevant role in DFTD transmission. Moreover, Tasmanian devils have a promiscuous mating system (Russell 1984; Owen and Pemberton 2005) and litters with multiple paternity are very common in the wild (Menna Jones, unpublished data), which further suggests that mixing preferences in this season are somewhat heterogeneous. As it was not possible to relate the few individuals with higher values of other important network metrics to any sex or age group there is no scope to design disease control strategies targeted at particular demographic groups. This highlights the point that although it is important to investigate the possible effects of superspreaders in disease dynamics (Galvany and

May 2005) and the network structure of host species (Bansal et al. 2007), translating this information to the delivery of effective disease control actions in wildlife is not always possible.

There is broad consensus that the key epidemiological parameter to determine the likelihood and extent of an epidemic is the basic reproductive number, R_0. Most field epidemiological studies derive the results of their models in terms of R_0 as it allows the definition of a theoretical framework from which to forecast disease outbreaks and intervention methods. The critical value at which an epidemic can occur ($R_0 > 1$) has been shown to depend on the dynamics of transmission and recovery rates as well as on the structure of the host population (Diekmann and Heesterbeek 2000). Calculating R_0 in wild animal populations has always been a challenging task as it requires a detailed knowledge of the contact heterogeneities and host population structure. The results obtained from this study show that the disease simulation outbreaks with contact network heterogeneities had a modest effect on estimation of the transmissibility threshold for $R_0 > 1$ compared to the mean field compartmental model. However, several empirical studies in human and wildlife diseases have shown that mean number of contacts alone can produce unreliable estimates of R_0 (Anderson et al. 2004; Meyers et al. 2005; Porphyre et al. 2008; Volz and Meyers 2009). This emphasises that there are no simple contact rate measures in wild populations or standard assumptions of transmission rate in host-pathogen systems and therefore, estimating the effect of contact heterogeneities in epidemic models is a critical step for determining R_0 and assessing possible disease control policies.

An important caveat when growing networks and using disease simulation outbreaks is the effect of biological factors that can alter the average number of contacts

between individuals. For example, host density, host total population size, carrying capacity or habitat constraints may have an effect on contact rates and therefore on the structural properties of networks and estimates of R_0 (Porphyre et al. 2008; Perkins et al. 2009). In addition, the incubation, latent and infectious periods of the disease and heterogeneities in individual susceptibility have been shown to have a significant effect on estimates of R_0 and on temporal disease dynamics (Anderson and May 1991; Keeling et al. 2003). Although the network simulation model in this study included carrying capacity, sex and seasonal variation in contact rates, it was not possible to include other important host and pathogen parameters such as heterogeneities in susceptibility to acquiring infection, virulence and disease induced mortality. Given the high virulence of DFTD and the strong selective pressure for any traits that could decrease mortality, there is scope for adaptive responses between the devil and the tumour. Indeed, the low impact of DFTD in the northwest of Tasmania at the West Pencil Pine population reported in this study (Chapter 4), suggests that differences in susceptibility to acquiring DFTD could result in disparate values of R_0. The reliability and accuracy of the estimates of R_0 should increase as new information on latent period of the disease and a diagnostic test for DFTD become available. Likewise, any information on the relationship between tumour strain, degree of tolerance and the effect of immuno-genetic diversity on disease susceptibility/resistance may help to obtain better estimates of disease dynamics and to assess possible management strategies for the long term conservation of Tasmanian devils.

Immuno-genetic heterogeneities and host-pathogen adaptations: understanding transmission dynamics and managing disease threats from an evolutionary perspective

Understanding the epidemiology of DFTD and its impact on wild devil populations provides a basic framework from which management strategies and their possible outcomes can be assessed. To date, studies that had observed and assessed the natural progression of DFTD in wild populations have reported significant negative impacts on the life-history traits, demographic composition, population size, and population genetic structure of Tasmanian devils (Lachish, et al. 2007, 2009, 2011; Jones et al 2008). In addition, the transmission dynamics of DFTD and the high disease prevalence in affected populations, even after they have been reduced by up to 90%, suggest that DFTD can lead to diseased-induced extinction (McCallum et al. 2009). By contrast, this study has revealed that the impact and epidemiology of DFTD as it moves for the first time into the different northwestern genetic provenance is significantly lower than in all the eastern populations for which similar epidemiological data are available.

Three plausible explanations for the lack of population impacts and low infection rates at the western genetic population are proposed in this study: i) a lower degree of susceptibility to acquiring infection due to different MHC genotypes; ii) a decrease in tumour virulence in the tetraploid strain of DFTD at the West Pencil Pine population; and iii) a behavioural facultative response of the host to reduce contact rate. However, this study also demonstrates that biting patterns between western and eastern populations are not significantly different (Chapter 5). This suggests that the

differences in DFTD impact between eastern and western populations are due to MHC genotypic differences, tumour virulence or a combination of both.

There has been increasing evidence of the functional importance of MHC variability in host responses to parasitic and pathogenic threats and its relevance for evolutionary ecology and conservation (Sommer 2005; Acevedo-Whitehouse and Cunningham 2006; Radwan et al. 2010). Several studies in natural populations have highlighted significant relationships between MHC diversity and parasite burden. For example, MHC heterozygosity in mole rats (*Spalax ehrenbergi*) is highest in geographic areas with high moisture and humidity where infectivity of mites and endoparasitic helminths is also higher (Nevo and Bieles 1992). Likewise, resistance and susceptibility to gastro-intestinal parasites has been demonstrated to strongly depend on MHC diversity in Soay sheep (*Ovis aries*) (Paterson et al. 1998), yellow-necked mouse (*Apodemus flavicollis*) (Meyer-Lucht and Sommer 2005), three-spined stickleback (*Gasterosteus aculeatus*) (Wegner et al. 2003) and mouse lemur (*Microcebus murinus*) (Schad et al. 2005). These studies suggest that while MHC diversity has a critical role in parasite resistance, MHC variability also reflects rapid adaptive and evolutionary processes between host and pathogens. Trade-offs in immune responses are assumed to be costly to the host, but the mechanisms underlying those costs are not always consistent among populations and may interact with ecological processes (Lochmiller and Deerenberg 2000; Sandland and Minchella 2003). Furthermore, heterogeneities in host tolerance and pathogen virulence usually respond to adaptive and evolutionary processes that are difficult to pinpoint but are of paramount importance for understanding disease dynamics and host-pathogen evolution (Ebert and Bull 2003; Read et al. 2009; Raberg et al. 2009)

Studying the interaction between the Tasmanian devil and its novel tumour provides the first opportunity to observe rapid evolutionary processes in a transmissible cancer. My results provide the first indications that devil genotypes or tumour strains might be affecting the epidemiology of DFTD, although I have not specifically tested for a relationship between host MHC diversity and susceptibility/resistance to DFTD infection or for the effect of tumour strains on host tolerance or tumour virulence. The disease has, for the first time, encountered hosts of a divergent genetic subpopulation from the index case and with disparate MHC genotypes. Whether DFTD is capable of affecting these hosts or if the tumour is evolving into a less virulent form is a subject of further study. This study system provides, nonetheless, the first opportunity to study the rapid evolution of a contagious cancer and its host in the wild. Study of host-pathogen systems in natural populations allows assessment of Darwinian selection acting in an ecological context over short time scales, which can increase the potential for identifying sources of selective pressure (Sandland and Minchella 2003; Piertney and Oliver 2006). This widens the capacity for both, improving the scope and efficacy of management strategies for conservation, and further investigating the effect of host genetic structure and pathogen strains in coevolutionary or adaptive processes (Ebert and Bull 2003).

Potential directions for future research

This thesis has highlighted the importance of obtaining and analysing empirical data on host behaviour and disease dynamics for understanding the ecology of host-pathogen systems and the impact of disease on host populations. Understanding adaptive and coevolutionary processes in a contagious cancer in the early stages of its emergence is a major area for future research. This opportunity is uniquely provided by the devil-DFTD system. The integration of disease ecology, ecological immunology and evolutionary epidemiology is nowadays considered crucial for predicting and understanding the role of pathogens in conservation biology (Hudson et al. 2002; Galvany 2003; Hawley and Altizer 2011). In addition, incorporating cross-disciplinary collaboration between the fields of immunology, genetics, epidemiology and mathematical modeling will advance fundamental research in areas such as immuno-competence, adaptive and coevolutionary processes in host-pathogen systems.

As suggested in this thesis (Chapter 4), further immuno-genetic studies are urgently needed to clarify if there are particular genotypes less susceptible or resistant to DFTD and to determine the frequency of the MHC types in the entire population. This will help to test null hypotheses concerning the relationship between genetic structure and infection outcomes in natural host-pathogen systems. In addition, understanding how pathogens evolve to become more or less harmful and their long term impact on host populations is of paramount importance for conservation and evolutionary biology (Ewald 1994; Cleaveland et al. 2003). In that sense, identifying possible interactions between genetic structure of both the devil and DFTD and virulence-

transmission relationships should help to detect the strength and direction of the adaptive processes that favours coexistence of host and pathogens (eg. de Roode and Altizer 2010). Further research also should aim to obtain long-term longitudinal data to assess how natural selection acts on host-pathogen traits or phenotypic plasticity and its consequences for possible ecological trade-offs and adaptive responses (Rohani and King 2010). Host and pathogens usually face many shifting challenges over evolutionary time and the extent to which pathogens overcome immune detection and hosts resist or adapt to infectious agents determines whether they will successfully coexist (Bowers et al. 1994). DFTD has evolved into eight different tumour strains since it first detection in 1996 (Ann Maree Pearse, unpublished data). Determining the rate of mutation in the tumour and its consequences for possible immune and adaptive responses in the host is another area that deserves urgent research.

Epidemiological models in wildlife diseases have been increasingly used to foresee the impact of pathogens in wild populations and to determine the effect of possible interventions and management actions. However, epidemiological studies almost inevitably have to deal with several uncertainties in the host-pathogen system. Fitting immunological dynamics and evolutionary processes to epidemic models, parameterising spatio-temporal data, contact heterogeneities and biological complexities into mathematical models are standing challenges where further advances are urgently required (Hudson et al. 2002; Galvani 2003). For example, understanding how pathogen exposure/strain affects immune variation or disease tolerance is critical to testing assumptions of host-pathogen evolution (Carval and Ferreire 2010). The use of epidemiological models that can capture the effect of

multi-strain complexities and its interactions with host immunological and susceptibility status will expand the scope for evaluating and understanding coadaptive processes in Tasmanian devils and DFTD. In that sense, efforts should be focused on bridging the gap between theoretical models and empirical data, balancing mathematical tractability and biological realism.

A key issue in modern epidemiology is the existence of 'superspreaders', those hosts that are responsible for a disproportionately high number of secondary infections (Lloyd-Smith et al. 2005). The presence and effect of superspreading should be addressed, however, within a framework that integrates disease ecology and evolutionary epidemiology. Field experiments need to be undertaken to test whether the behaviour of diseased individuals could be related to superspreading and to which extent this is pathogen specific. More importantly, tolerance may also lead to production of superspreaders. Individuals with increased tolerance to pathogens or less virulent pathogen strains can result in higher transmission rate, because they increase their infectious time within the population (Soper 1907). This highlights that the mechanisms of superspreading not only depend on host behaviour but also interact with adaptive responses and coevolutionary processes between host and pathogens (Miller et al. 2006).

The emergence of DFTD and its sudden impact on wild populations coupled with the ecological and epidemiological adaptive responses of the devil and the tumour present a unique study system for understanding and managing the impact of emerging diseases at an ecosystem level. Ecological perturbations such as diseases can play a key role in the regulation of an ecosystem and its function (Holdo et al. 2009). The

devil is the top predator of the Tasmanian ecosystem, thus, its sudden demise also has broader conservation implications (Soule et al. 2005). Top predators are highly interactive species which can drive ecosystem dynamics via shifts in predator-prey dynamics and trophic cascades (Beschta and Ripple 2009; Letnic et al. 2009). Therefore, future management strategies should also assess the consequences and cascade effects of devil population declines, aiming to restore and maintain the ecological function of this top-order predator.

Scope for improving the management DFTD in the light of this study

Control actions for managing wildlife diseases in threatened species are usually difficult to implement (Woodroffe 1999), especially for emerging diseases with a genetic origin and high virulence such as DFTD. In response to the growing concerns of the sudden devil population declines after the DFTD epidemic outbreak the Tasmanian Department of Primary Industries, Parks, Water and Environment formed the "Save the Tasmanian Devil Program" (STTDP) (www.tassiedevil.com.au). Early in its inception, a major goal for the STTDP was to maintain the Tasmanian devil as an ecologically functional species through a multi-faceted management plan (Jones et al. 2007). However, *in situ* management options to mitigate the impact of DFTD in wild devil populations are limited. Selective culling of infected individuals in a semi-isolated peninsula failed to eradicate DFTD from the population in question (Lachish et al. 2010). The development of a vaccine or treatment for the disease in the wild seems unlikely, at least in the short term, given that DFTD is an infectious cancer, and the few vaccines that have been developed for cancers target a virus that predisposes

individuals to developing cancer (but see Woods et al. 2007). As a consequence, most current management actions to deal with the threat of DFTD have been focused in *ex situ* conservation programs aimed at building insurance populations from which reintroductions could be undertaken following extinction of the devil and thus also its tumour in the wild.

The insurance metapopulation aims to manage between 1,500 (entirely intensive captive) and 5,000 (entirely semi-wild) devils, derived from 150 wild-caught founders, for 30 years for 95% retention of current wild genetic diversity. It comprises intensive captive facilities, free-range enclosures which promote more natural behaviours and therefore devils that are more suitable for reintroduction, as well as semi-wild populations on fenced peninsulas and islands. Methods for maintaining and breeding devils in free-range enclosures are being developed. Large landscape scale fencing is expensive and vulnerable to bio-security breaching (but see Bode and Wintle 2008). However, it is used extensively in New Zealand for predator control for wildlife conservation (Brown 1994; Wilson et al. 2007) and in southern Africa for veterinary disease control to separate livestock from wildlife (Bruckner et al. 2002). The results from this study, that indicate slower disease progression as the tumour enters the genetically different population in northwest Tasmania due either to some degree of host tolerance/resistance, a less virulent tumour or an interaction between the two, opens the possibility of new management prospects for recovering the Tasmanian devil as an ecologically functional species in the wild.

This study has provided evidence of disparate epidemiology and impact of DFTD in two different genetic provenances. The results of Chapters 4 and 5 coupled with

evidence of tumour evolution, translated into eight different DFTD strains, (Anne-Maree Pearse, unpublished data), offer new insights and avenues to be tested for managing this extinction threatening disease. Management options can now be characterized in four possible scenarios:

1.- *Reintroductions from captive insurance populations after extinction of host and tumour in the wild.* Although a few high profile cases demonstrate that captive breeding programs can play an important role in preventing extinctions in the wild (Kleiman and Rylands 2002; Cade and Burnham 2003), most reintroductions from captive populations have failed in their goal of re-establishing populations after natural extinctions (Griffith et al. 1989; Wolf et al. 1996; Jule et al. 2008; Bowkett 2009). Nonetheless, given the risk of disease-induced extinction in the Tasmanian devil (McCallum and Jones 2006; McCallum et al. 2009), a captive insurance metapopulation must form a critical component of a comprehensive management plan aimed at enhancing the conservation prognosis of the species, including the possibility of contributing to the genetic rescue of small wild populations in the future.

2.- *Gradual recovery through natural coevolution between the host and the pathogen.* Understanding host-pathogen interactions and coevolution might be critical for the long-term conservation of Tasmanian devils. With 100% mortality of infected devils within 6-12 months after the presence of clinical signs, selective pressure on devils for any traits that may confer disease tolerance or resistance at an individual or a population level, is extremely strong. However, the time frame over which selective host-pathogen

coadaptation processes might achieve coexistence is unknown. The only case of a transmissible cancer that could have evolved into a non-lethal form is the canine transmissible venereal tumour (CTVT). Recent studies into the genetic ancestry of CTVT (Murgia et al. 2006; Rebbeck et al. 2009) suggest that the tumour cell line was probably founded by a single wolf between 7800 and 78000 years ago. Compared to CTVT, DFTD is a young tumour, and although the recognition of at least eight different strains detected in the wild so far (Ann Maree Pearse, submitted) suggests that the tumour is evolving, the extent to which this evolution might be adaptive is not known. It is not possible at this stage to foresee if natural coexistence between the devil and the tumour is a likely outcome. Furthermore, the likelihood for coexistence 50 years after disease arrival in disease simulation network models undertaken in this study (Chapter 3) was particularly low and strictly subject to a long latent period. However, as discussed in Chapter 3, these models do not take into account variability in critical disease parameters (eg. infectious period, tumour strain or MHC genotypic differences) with each generation of infection, which can drastically affect the transmission dynamics of the disease and the prognosis for host-pathogen coexistence.

3.- *Management of disease resistance and/or virulence.* The low infection rates and lack of DFTD population impacts in northwestern Tasmania reported in this thesis and the potential effect of host genotype in disease dynamics widens the scope for *in situ* management strategies to deal with the threat of DFTD in the wild. If the reduced effects of the epidemic in northwest Tasmania are due to a genetically-based disease resistance/tolerance, then

establishing those northwestern MHC genotypes in previously ravaged eastern populations through wild to wild translocation could, in theory, enhance the immune response of devils and slow the progression and effect of DFTD in the wild. This strategy would not be viable, however, if a slower growing tumour strain were primarily responsible for the reduced impacts of DFTD in northwest Tasmania. In this scenario, translocating a slower-growing tumour, which could potentially outcompete the more virulent and deadly strains might be an option of managing the virulence of DFTD (Ebert and Bull 2003). Host/pathogen systems are expected to coevolve towards increased resistance in the host and optimal pathogen virulence – a pathogen that allows its host to live long enough for maximal transmission. While DFTD is an infectious disease, it is also a cancer originated from a single cell line. The clonal cell line could be expected to accumulate deleterious mutations according to the principle of Muller's ratchet (Muller 1932), which could lead to further fitness deterioration. The trade-offs and evolution between virulence and transmission should be assessed across DFTD strains and host genotypes. This provides a promising avenue to explore, which can improve prospects for developing adaptive management strategies in the devil-DFTD system and further assessing rapid evolutionary changes in virulence of DFTD.

4) *Genetic rescue of Tasmanian devils for future disease resilience*

Genetic restoration in endangered species has been critical for their management and long-term survival (Frankham et al. 2002). Indeed, it has been demonstrated that genetic factors have a significant role in extinction processes in a broad range of taxa (Spielman et al. 2004). Tasmanian devils

have already lost about half of their genetic diversity (Jones et al. 2004) and there is preliminary evidence that a selective sweep affected MHC Class I genes in eastern Tasmania (Siddle et al. 2010). Mixing eastern and western genotypes would, in principle, increase the overall diversity of devils as well as building higher resilience to future environmental challenges and diseases.

Whilst reintroductions from captive breeding programs have been widely used as a management tool for species threatened with extinction (Bowkett 2009), this option does not necessarily meet the goal of maintaining the Tasmanian devil as an ecologically functional species. Likewise, a gradual recovery through natural coevolution between the devil and the tumour is uncertain as there is no evidence that populations ravaged by DFTD have recovered and the basis for the disparate patterns in disease susceptibility in the northwest are not yet understood. Assisted devil genetic restoration, on the other hand, involves a pro active management action. Even if the patterns of disease progression in the northwest are driven by tumour strain, genetic rescue is a valuable goal that could increase resistance to future threats to Tasmanian devils, including disease, climate change and others (Hawley and Altizer 2011). Genetic management in the context of both controlling infectious diseases (Bishop and MacKenzie 2003; Romanov et al. 2009) and using wild translocations to increase genetic diversity (Lambert et al. 2005; Brekke et al. 2011) have been proved to be critical for the long-term conservation prospects of endangered species. In the case of Tasmanian devils, two potential benefits can be visualized from genetic rescue, even if this needs to be implemented in the first instance in the more closely managed insurance metapopulations. First, increasing genetic diversity and fitness and reducing inbreeding. A significant increase in relatedness has been observed in

populations where DFTD has caused dramatic population declines (Lachish et al. 2011). Therefore translocating northwestern genotypes into these affected populations could restore the genetic diversity and the overall fitness of populations affected by DFTD. Second, if resistant or more tolerant MHC genotypes can be found in the northwestern populations, these could be established in eastern genotypes with the aim of reducing the impact of DFTD. This is assuming that there is heritable disease-resistance in Tasmanian devils. To date, there is no conclusive evidence of an association between MHC genotype and disease susceptibility. Clearly, this is an area that needs future work. Research into the relationship between genetic structure and DFTD resilience in West Pencil Pine is currently in progress and its results will provide an important breakthrough for testing potential management strategies.

A possible setback of using wild translocations to increase genetic diversity or disease resilience is the prospects of causing outbreeding depression, leading to a genetic parental incompatibility due to the production of phenotypes with less local adaptations (Shields 1993). This was observed in an experimental study in the common frog (*Rana temporaria*) to test the potential effects of translocating wild frogs in areas affected with population declines, in which one small isolated population was artificially crossed with a large well connected population (Sagvik et al. 2005). Offspring of females from the large population showed smaller hatchling size and higher incidence of malformation when fathered by the small population (Sagvik et al. 2005). Although outbreeding depression is not common in natural populations (Frankham et al. 2011) and has been found mostly in invertebrates with intraspecific mating systems (i.e. Alstad and Edmunds 1983; Brown 1991) it has been observed in severely inbred populations of birds (Marr et al. 2002) and mammals

(Turcek 1951). Given that Tasmanian devils do not show severe inbreeding and that, although limited, there has been natural gene flow between east and west populations the risk of outbreeding depression as a result of northwest to east translocation is low (Frankham et al. 2011).

The different epidemic patterns found at West Pencil Pine in the northwest of Tasmania, where a tetraploid strain of DFTD is coupled with the longer survival in diseased individuals compared with an eastern population, raises further insights. First, it is conceivable that the slow progression of DFTD at West Pencil Pine is due to the tumour itself rather than the immune response of the host or its MHC genotype. Tetraploid cells have slower growth rates compared to diploid cells in tumorigenesis (Margolis et al. 2003), thus, the slow progression and reduced impact of DFTD at West Pencil Pine could be attributed to a delay effect caused by the slow tumour growth. Whether a tetraploid tumour with a slower growth rate would have a longer incubation period is not known. A tetraploid strain, however, would not discriminate between individuals which it is more likely to infect. Results from Chapter 4 show that unlike all eastern populations the age structure at West Pencil Pine remains unaltered, having a large proportion of old (3-5 years old) individuals. This means that those individuals have coexisted with other infected devils for up to four years without acquiring infection (almost all the adult life of a devil). In addition, the transmission dynamics of DFTD have been proven to be driven mainly by 2-3 and 3-4 year old devils in all populations for which data are available (McCallum et al. 2009), resulting in populations biased towards young individuals (Jones et al. 2008; Lachish et al. 2009). This suggests that there is something very unusual happening at West Pencil Pine, where 3-5 year old devils form more than 50% of the total population.

Second, the outcome of host pathogen interactions is usually towards optimal virulence of the pathogen with host persistence (Ebert and Bull 2003). A pathogen that kills its host too quickly will have lower fitness than one of lower virulence. There is the possibility that the devil tumour is evolving into a less virulent form as was the case with CTVT and many other diseases (Belov 2010). For example, the initial effects of myxomatosis, a viral disease introduced as a biological control agent to reduce rabbit (*Oryctolagus cuniculus*) populations, were dramatic, resulting in rapid death of most infected rabbits and leading to major population declines (Ross and Sander 1987). Several years later the original strain became very rare and several new strains became dominant, which led to a higher genetic resistance in rabbit populations (Ross and Sander 1987). The initial strain of DFTD has now disappeared from wild devil populations but strains 1 and 2 have evolved into tetraploid strains, the latter being the strain now found at West Pencil Pine (Ann-Maree Pearse, unpublished data). The effects of the tetraploid versus non-tetraploid strains of DFTD at the host population level and their possible relationship with susceptibility, virulence and MHC genotypes could be tested experimentally by following the growth of tumours and their physiological impact on captive individuals with different genotypes. Transmission trials aimed at showing if tumour strain or MHC genotype have uniform effects on hosts should become a priority and would assist in directing future management strategies.

Directly transmissible cancers are rare, however, their genetic nature and inherent instability coupled with the interactive factors that drive tumorigenesis and transmission (eg. environmental exposure to carcinogens, low genetic diversity) make

the management of this type of disease particularly difficult (McAloose and Newton 2009; McCallum and Jones, in press). Because cancers in wildlife are increasingly being regarded as a conservation threat (McAloose and Newton 2009), understanding the aetiology and biology of wildlife cancers and its implications for population dynamics and host-pathogen evolution is a growing challenge. Competition and coexistence in host-parasite systems play a fundamental role in transmission and population dynamics (Bowers et al. 1994). Virulence is a fundamental trait of pathogen life history and the result of complex interactions between ecological, evolutionary and epidemiological processes (Ebert and Bull 2003; Galvani 2003). Including tumour strain dynamics in devil-DFTD epidemic models should help to further investigate effects of virulence in the epidemiology of DFTD. Likewise, identifying adaptive and selective processes and their interaction with host life history and host behavioural ecology should help to predict possible epidemic outcomes and direct management strategies for these type of wildlife diseases.

This study highlights the usefulness of obtaining longitudinal data sets and consistent monitoring of the natural progression of a disease in a host-pathogen system for interpreting wildlife disease dynamics and refining adaptive management actions. Few studies in natural populations, particularly in vertebrates, can assess evolutionary ecology and epidemiological theory *in situ* (Galvany 2003). As suggested in this study, further work is needed to develop a more integrative understanding of how potential rapid evolutionary processes, behavioural and ecological host-pathogen interactions impact infection dynamics and epidemic outcomes. Evolutionary epidemic models will also assist in testing the effects of heterogeneities on critical disease parameters in the dynamics and impact of DFTD and in predicting the

conservation prospects of the species. This will also motivate the development of cross-disciplinary studies, closing the gap between field, laboratory and theoretical research, addressing the long-term threat of disease in natural populations and better understanding the mechanisms involved in transmission.

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Appendices 1 and 2 have been removed for copyright or proprietary reasons.

Appendix 1

Jones, ME et al, 2007, Conservation Management of Tasmanian Devils in the Context of an Emerging, Extinction-threatening Disease: Devil Facial Tumor Disease, EcoHealth 4, 326–337, DOI: 10.1007/s10393-007-0120-6

Appendix 2

Jones, ME et al, 2008, Life-history change in disease-ravaged Tasmanian devil populations, Proceedings of the National Academy of Sciences, 105 (29) , 10023–10027, DOI: 10.1073/pnas.0711236105

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Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease-induced extinction

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Abstract. Most pathogens threatening to cause extinction of a host species are maintained on one or more reservoir hosts, in addition to the species that is threatened by disease. Further, most conventional host–pathogen theory assumes that transmission is related to host density, and therefore a pathogen should become extinct before its sole host. Tasmanian devil facial tumor disease is a recently emerged infectious cancer that has led to massive population declines and grave concerns for the future persistence of this largest surviving marsupial carnivore. Here we report the results of mark–recapture studies at six sites and use these data to estimate epidemiological parameters critical to both accurately assessing the risk of extinction from this disease and effectively managing this disease threat. Three sites were monitored from before or close to the time of disease arrival, and at three others disease was well established when trapping began, in one site for at least 10 years. We found no evidence for sex-specific differences in disease prevalence and little evidence of consistent seasonal variation in the force of infection. At all sites, the disease was maintained at high levels of prevalence (>50% in 2–3-year-old animals), despite causing major population declines. We also provide the first estimates of the basic reproductive rate R_0 for this disease. Using a simple age-structured deterministic model, we show that our results are not consistent with transmission being proportional to the density of infected hosts but are consistent with frequency-dependent transmission. This conclusion is further supported by the observation that local disease prevalence in 2–3-year-olds still exceeds 50% at a site where population density has been reduced by up to 90% in the past 12 years. These findings lend considerable weight to concerns that this host-specific pathogen will cause the extinction of the Tasmanian devil. Our study highlights the importance of rapidly implementing monitoring programs to determine how transmission depends on host density and emphasizes the need for ongoing management strategies involving a disease-free "insurance population," along with ongoing field monitoring programs to confirm whether local population extinction occurs.

Key words: basic reproductive number R_0; conservation biology; extinction; facial tumor disease; pathogen transmission; Sarcophilus harrisii; *Tasmanian devil.*

Introduction

Population decline caused by infectious disease is increasingly being recognized as a major threat to the survival of some species (McCallum and Dobson 1995, Lafferty and Gerber 2002, de Castro and Bolker 2005, Hoffmann et al. 2008). Recent examples of infectious disease threatening the extinction of endangered species include the Ebola virus affecting western gorillas (Leroy et al. 2004), the fungus *Batrachochytrium dendrobatidis* in numerous frog species worldwide (Berger et al. 1998, Lips et al. 2006), rabies in Ethiopian wolves (Randall et al. 2006) and African wild dogs (Vial et al. 2006), and avian malaria and birdpox threatening Hawaiian land birds (van Riper et al. 1986, 2002). In all these examples, the pathogen in question has a broad host range, including one or more reservoir species on which the disease can be maintained at high prevalence, even as the threatened species decline towards extinction.

The Tasmanian devil, *Sarcophilus harrisii*, the largest surviving marsupial carnivore, has suffered massive population declines as a result of Tasmanian devil facial tumor disease (hereafter DFTD), a recently emerged novel infectious cancer (Hawkins et al. 2006, McCallum et al. 2007, Jones et al. 2008, McCallum 2008). First reported in northeastern Tasmania in 1996, DFTD has spread south and west over the majority of the geographic range of the species, leading to a total population decline of >60%, with declines in excess of 90% in the Northeast, where it has been present for the longest time (Hawkins et al. 2006, McCallum et al. 2007). The disease is an infectious cancer, spread as a transmissible cell line (Pearse and Swift 2006). This unusual infection process is thought to be possible

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because of the extremely low genetic diversity of the devil population, particularly in the Major Histocompatibility Complex (MHC), the vertebrate immune system genes closely associated with self–nonself recognition. All individuals examined by Siddle et al. (2007) from the eastern part of the state possessed very similar MHC genotypes, which were described as "functionally identical." The recent emergence of DFTD and its intimate genetic relationship with Tasmanian devils make it highly unlikely that there are alternate hosts, and no evidence of the disease in any other species has been observed (Hawkins et al. 2006, McCallum 2008).

Tasmanian devils bite each other frequently during sexual encounters and interactions over food; adults are most commonly bitten around the head region (Hamede et al. 2008). It is highly likely that biting is the primary means of tumor transmission, given that tumors are almost invariably first observed on the head or face (Loh et al. 2006, Pyecroft et al. 2007). However, transmission through uninfected animals scavenging on the carcasses of other devils that have died from the disease or through infected and susceptible devils feeding on the same prey carcass cannot be entirely discounted.

The incubation period of DFTD is currently unknown. The infection process through transfer of viable cancer cells suggests that transmission is unlikely to occur before visible tumors around the face and mouth are present. This implies that the latent period (the time from acquisition of infection to infectiousness) is at least as long as the incubation period (the time between infection and first appearance of clinical signs). There is one anecdotal record of an individual held in captivity that first developed disease 10 months after its removal from an infected population, and trapping records (Tasmanian Department of Primary Industries and Water [DPIW], *unpublished manuscript*; M. E. Jones, *unpublished data*) suggest that tumors develop from small nodules to large friable tumors over a period of 2–3 months. Once a visible tumor is present, the disease is typically fatal within 6 months (Hawkins et al. 2006).

Greatly reduced survival and population growth rates in disease-affected populations indicate that population declines could lead to local extinctions within 15 years of disease arrival (Lachish et al. 2007). In addition, estimates of disease spread indicate that DFTD will cover the entirety of the devils' range in as little as 5 years time (McCallum et al. 2007). Extrapolation of these observed trends has led to concerns that this novel disease may lead to the extinction of Tasmanian devils in the wild in the next 25 to 30 years (McCallum et al. 2007).

According to conventional host–pathogen theory, however, a single-host pathogen should become extinct before its sole host because transmission is usually dependent on host density. Below a threshold density, transmission is reduced sufficiently that the pathogen cannot be maintained within the host population (Anderson 1991). Only if pathogen transmission is independent of host density can a single-host pathogen in itself lead to the extinction of its host (de Castro and Bolker 2005). Hence, to determine the risk of extinction to the Tasmanian devil population from DFTD, we require information on the relationship between transmission and host population density.

A thorough understanding of the relationship between transmission and host density is also needed for the effective management of infectious diseases. Eradication of any infectious disease from a population requires reducing the effective reproductive number R_e to below one at very low disease prevalence. In almost all situations, this requires driving the basic reproductive number R_0 (the mean number of secondary infections per primary infection in a fully susceptible population) to below one (Anderson and May 1991, Roberts 2007). Thus, estimating R_0 has been an essential component of managing recent disease threats, whether to human populations (for example, SARS [Anderson et al. 2004]) or to livestock (for example, foot and mouth disease [Ferguson et al. 2001]). To evaluate the feasibility of potential control strategies such as vaccination or disease suppression by culling of infected animals, it is critical to estimate R_0 in field situations and to determine how it might be affected by host density or population size. Estimating R_0 and understanding transmission dynamics in wildlife present a range of challenges beyond those in domestic animal or human populations. All individuals cannot be counted or examined, and contact tracing, as was done with SARS (Lipsitch et al. 2003), is essentially impossible. It is not surprising, therefore, that few estimates of R_0 in wildlife exist for any disease of importance for conservation (Lloyd-Smith et al. 2005, Real and Biek 2007).

In this paper, we use data derived from extensive mark–recapture studies at a number of sites throughout Tasmania to derive estimates of disease prevalence and the basic reproductive number, and to investigate the role of host density in disease transmission. This information is combined with previously published estimates of demographic parameters (Lachish et al. 2007, 2009) to develop simple deterministic SEI models to evaluate the likelihood of this disease leading to the extinction of the Tasmanian devil. By explicitly considering transmission dynamics, this approach provides a more reliable prognosis of extinction than the extrapolations of observed trends in McCallum et al. (2007) and Lachish et al. (2007).

Methods

Trapping and data collection

Tasmanian devils were trapped at six sites, shown on Fig. 1. One site, Freycinet, was trapped from 1999 onward as part of a life history study by M. E. Jones and students, with disease first detected in the area in 2001 (Lachish et al. 2007). A second site, Fentonbury, was trapped from just prior to any evidence of disease emergence. A third site, Wisedale, was first trapped in 2006, soon after local disease emergence: disease was

known to be present south of the site (Hawkins et al. 2006), but had not been detected 8 km north of the site in 2 years of trapping from 2004 (C. Hawkins and R. Hamede, *personal observations*). At the three remaining sites, Mt William, Bronte Park, and Buckland, disease was well established at the time trapping commenced, although a three-year mark–recapture study had been undertaken at Mt William in the 1980s (Pemberton 1990), well before emergence of the disease. Disease has been present at Mt William since at least 1996. High-quality photographs of DFTD-like signs in devils at Mt William were provided by Christo Baars in 1996 (Hawkins et al. 2006), and DFTD was confirmed in a tumor sampled in 1997 near Waterhouse, 45 km from Mt William (Loh et al. 2006; T. Knox, *personal communication*). Devils with DFTD-like signs were caught at Buckland in 1999 (M. E. Jones, *unpublished data*), though the disease was not confirmed there until 2005. DFTD signs were first observed and confirmed at Bronte in 2003, and our limited understanding of the history of spread of the disease suggests that the disease likely emerged there in 2001 or 2002 (McCallum et al. 2007).

Standardized trapping protocols were followed. At all sites other than Freycinet, 10-night trapping sessions were undertaken with 40–50 traps set over an area of 25–30 km^2 (see Hawkins et al. 2006). Field work scheduling meant that trapping frequency and timing differed between sites and years: all were sampled between two and four times per annum. At Freycinet, consecutive 7-night trapping sessions were conducted with 25–35 traps set over each of four 35–50 km^2 sections comprising the entire 160-km^2 peninsula (see Lachish et al. 2007). Each section was trapped up to four times per year.

All individuals trapped were individually tagged, using microchip transponders (individual ear tattoos were used prior to 2004 at Freycinet), weighed, sexed, measured, assessed for disease status, and released at the point of capture. Currently, DFTD can only definitively be confirmed through histological examination of a biopsied tumor. The likelihood of an individual having DFTD was therefore scored on the basis of the external morphology of any lumps or lesions found using an index ranging from 1 (no lumps or lesions found), through 2 and 3 (lumps or lesions unlikely to be DFTD), to 4 and 5 (characteristic DFTD tumors present [Hawkins et al. 2006]). Only individuals with lesions or lumps characteristic of DFTD (those that scored 4 or 5) were included as diseased in these analyses.

To examine seasonal trends, we grouped months into summer (late November through February), approximately corresponding to the period in which juveniles become independent and disperse; autumn (March through May), during which mating and birth normally occur; winter (June through August) during which females have large pouch young; and spring (September through early November) when most young are in the den.

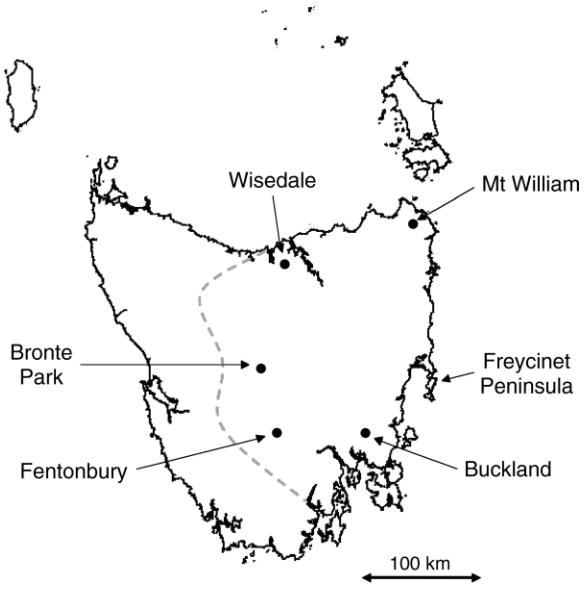

FIG. 1. Map of Tasmania, indicating locations of study sites. The approximate location of the disease front, as of early 2008, is shown with a dashed line (updated from Hawkins et al. [2006] and McCallum et al. [2007]).

Allocation into age classes

Breeding in Tasmanian devils is seasonal, with half of births occurring in March (the Austral autumn) after a short (3-week) gestation (Hesterman 2008). Pouch vacation, which is functionally equivalent to birth in placentals, occurs 15–16 weeks later (primarily in August). As is typical of marsupials (Cockburn and Johnson 1988), a large proportion of the year is devoted to reproduction, with weaning in devils occurring the following summer between December and February (Hesterman 2008). For the purposes of allocating animals into age classes, all births were assumed to occur on the mean birth date of 20 March each year. Animals were aged to their nearest year-class of birth using molar eruption, tooth wear indices, and canine over-eruption (distance from the dentine-enamel junction to the gum). This method is precise for aging devils to 3 years of age (M. E. Jones, D. Sinn, N. Beeton et al., *unpublished manuscript*). Accordingly, all animals with estimated ages of three or greater were pooled into a single age category.

Statistical analysis

Mark–recapture data were analyzed using MARK (Cooch and White 2002). Population size within the 7–10 day trapping trips at each site was estimated using closed population estimates including heterogeneity in capture probabilities with time and between individuals (Chao et al. 1992) implemented in the program CAPTURE (Otis et al. 1978, Rexstad and Burnham

1992). We also used results from these models to investigate whether the recapture probability within field trips was influenced by infection status. Population size estimates, together with 95% confidence bounds, were converted to estimates of density by dividing by the area of a minimum convex polygon constructed around the trapping grid, with an additional 2-km boundary strip (Kenward 1985) (representing approximately half the home range diameter of a Tasmanian devil [Pemberton 1990]), unless adjacent to a coastline, in which case the strip was applied only to any land boundaries.

Prevalence data were analyzed using logistic models implemented in R (version 2.6.1, R Development Core Team 2007). Because the residual deviance in logistic models suggested overdispersion, we used generalized mixed models, implemented using the function lmer in R package lme4, with the field trip–age class interaction as a random error term. As predictor variables, we explored age class (1–2, 2–3, 3+ years), season (austral winter, spring, summer, autumn), and trend (years from the first appearance of disease, or the start of the data set, whichever was later). We fitted models using all possible combinations of main effects and up to two-way interactions. We also explored models with aggregated versions of age class (1–2, 2+) and season (summer, other seasons), again with all possible combinations of main effects and up to two-way interactions. For each model, we calculated small sample corrected Akaike Information Criteria (AIC_c) and Akaike weights (see Burnham and Anderson 2004).

Estimation of R_0

We approximated the basic reproductive number R_0 from r_0, the rate of increase in prevalence per unit time following the first introduction of disease into the population using Eq. 1 (R_0^+ in Roberts and Heesterbeek 2007)

$$R_0 = \exp(r_0 T) \tag{1}$$

where T is the mean generation time of the disease or serial interval (i.e., the average time between acquisition of infection and the infection being passed on to another individual). This is the sum of the incubation period and the time from the disease becoming detectable to first transmission. In the absence of accurate information on the latent period, other than the single anecdotal report of 10 months, we explored a plausible range of generation times of between 3 and 12 months. The trend parameter, from logistic regression models of prevalence following the first detection of disease at the two sites (Freycinet and Fentonbury) that were trapped from the time of first disease onset, provided an estimate of r_0.

Models of transmission dynamics

To investigate whether the observed changes in disease prevalence and population density through time were consistent with either frequency-dependent or density-dependent disease transmission, we constructed a simple deterministic SEI (Susceptible, Exposed, Infectious) model with annual age classes, based on devil life history parameters estimated in Lachish et al. (2007). Transmission was parameterized to match the initial increases in prevalence observed at Fentonbury in this present study, and only animals older than one were assumed susceptible (see Hawkins et al. 2006, Lachish et al. 2007). In this simplified model, transitions through age classes and from exposed to infectious classes are assumed to occur at constant rates. The equations used were as follows:

$$\frac{dS_0}{dt} = \left(\sum_i b_i N_i\right)(1 - N) - \mu_0 S_0 - f(I,N)S_0 - S_1 \tag{2}$$

$$\frac{dS_i}{dt} = -\mu_i S_i - f(I,N)S_i + S_{i-1} - S_i \quad \text{for } i > 0 \tag{3}$$

$$\frac{dE_i}{dt} = \begin{cases} f(I,N)S_0 - (\mu_i + d)E_i - E_i & \text{for } i = 0 \\ f(I,N)S_0 + E_{i-1} - (\mu_i + d)E_i - E_i & \text{for } i > 0 \end{cases} \tag{4}$$

$$\frac{dI_i}{dt} = \begin{cases} dE_i - (\mu_i + \alpha)I_i - I_i & \text{for } i = 0 \\ dE_i - (\mu_i + \alpha)I_i + I_{i-1} - I_i & \text{for } i > 0. \end{cases} \tag{5}$$

Here, i represents age class (0, 1, 2, ...). The variables S_i, E_i, I_i, and N_i represent susceptible, exposed, infectious, and total numbers of devils of age i (all scaled relative to the carrying capacity) with I and N representing totals across age classes. The parameters are: b_i, age-specific birth rates; μ_i, age-specific disease-independent mortality rates; d, the rate of transition from exposed to infectious age classes; and α, the increment in death rate caused by symptomatic disease. Transmission is modeled by the function $f(I, N)$, which is βI for density-dependent transmission and $\beta I/N$ for frequency-dependent transmission.

Results

Effects of DFTD infection on capture probability

There was no evidence that infection status had any effect on capture probability within field trips. We compared closed population models in which capture probability was constant with models in which capture probability varied between infected and uninfected devils at Bronte (13 trapping sessions), Fentonbury (12 trapping sessions), and Freycinet (7 trapping sessions). In none of these 32 sessions did AIC values indicate that a model with separate capture probability for infected and uninfected devils was preferred, and in each case the 95% confidence interval for the difference in capture probability included 0. There were 13 sessions in which the estimated capture probability was higher for healthy animals and 19 in which the estimated capture probability was higher for diseased animals ($P = 0.38$ for H_0 of equal capture probability, exact binomial test).

TABLE 1. The influence of host sex on disease prevalence at six sites.

Site	AIC_c (−sex)	AIC_c (+sex)	AIC_c weight (−sex)	AIC_c weight (+sex)	M:F log-odds ratio	SE
Fentonbury	159.91	163.13	0.83	0.17	0.0591	0.326
Wisedale	107.77	111.12	0.84	0.16	0.117	0.413
Bronte	112.64	112.28	0.46	0.54	−0.835	0.409
Buckland	77.50	81.81	0.71†	0.03	−0.118	0.487
Mt William	117.43	119.64	0.49‡	0.16	0.580	0.427
Freycinet	236.49	238.80	0.76	0.24	0.242	0.253

Note: AIC_c and AIC_c weights for models including additive effects of field trip and age class, with and without a sex effect, are shown, together with the estimated log-odds ratio (and standard error) of being infected for males (M) relative to females (F).

† AIC_c weight for model with only age class is 0.25.

‡ AIC_c weight for model with only age class is 0.35.

We have therefore treated prevalence of disease in the trapped population as an unbiased estimate of prevalence in the population as a whole.

Prevalence in males and females

There was no evidence of consistent differences in disease prevalence between males and females. Table 1 shows results of adding a sex effect to logistic models predicting prevalence from field trip (which encompasses possible trend and season effects) and age class. At one site (Bronte) the AIC_c weight of the model including sex was slightly greater than that of the model without the sex effect. At all other sites, the AIC_c weight of models without a sex effect was substantially greater. Models including an interaction between sex and field trip were not supported at any site. At four of the six sites, the log-odds of being infected were greater in males than in females, with the reverse being the case at the other two sites. Estimated odds ratios for the sex effect were small at four of the six sites, with a marginally significant ($P = 0.04$) female bias in infection at Bronte and a substantial (but insignificant) point estimate of male bias in infection at Mt William.

Increases in prevalence following disease invasion

Fig. 2A, D, G shows the prevalence of DFTD in all animals older than 1 year captured at Freycinet. No diseased animals <1 year old were observed. Table 2 summarizes the results of generalized mixed models based on these data. There are four models with similar support, each including trend and an age effect on prevalence. Three of these contrast 1–2-year-olds with older animals. There is also some evidence that the rate of increase in prevalence may differ between age classes, with three of the four most strongly supported models including an interaction between age and trend. Evidence for a seasonal effect is weaker. Prevalence is lower in 1–2-year-olds than in older animals (Fig. 2).

Fig. 2B, E, H shows the increase in prevalence of DFTD since its arrival at Fentonbury. There is strong support for models including both trend and contrasting 1–2-year-olds with older animals (see Table 2), as was the case at Freycinet. However, there is also strong support for a seasonal influence on prevalence, with all the well-supported models including a term that contrasts summer with the other three seasons. As with Freycinet, the best-supported model suggested that the rate of increase in prevalence differed with age.

Interpretation of the results at Wisedale was hampered both by the short run of data (2 years) and also by the fact that prevalence was at >30% in 3+ year-olds at the time of the first survey (Fig. 2). Nevertheless, there is strong support for an increase in prevalence through time and of higher prevalence in adults (2 years +) compared with 1–2-year-olds. However, there was no evidence of seasonality at this site (Table 2).

There was strong support for a model in which the rate of increase in prevalence among 2–3-year-old animals differed between the three sites, but weak support for models including seasonality (Table 3). Inspection of the parameter estimates and 95% posterior density intervals for the best-supported model (Table 4) suggests that prevalence increased more slowly at Freycinet than at Fentonbury. (The interval for the trend : Freycinet interaction does not include zero.)

Prevalence at sites where disease was established

The Bronte, Buckland, and Mt William sites each had disease well established at the time of the first survey, with estimated prevalence in excess of 50% in 2–3-year-olds (Fig. 3). At Bronte and Buckland, there was no evidence of a trend in prevalence with time (Table 5). However, at Mt William, despite the fact that disease had been present in this population for at least eight years when surveys commenced, there was strong support for an increase in prevalence with time. At both Mt William and Bronte, there was strong support for models in which prevalence differed between age classes (either all three age classes or 1–2-year-olds vs. older animals) and with season, with evidence of an interaction between the two factors at Mt William. At Buckland, which had the least data, the null model (no effect of season, age class, or trend) was most strongly supported.

Seasonality

Fig. 4 shows parameter estimates for the effect of season (relative to summer) on prevalence in 1–2-year-old

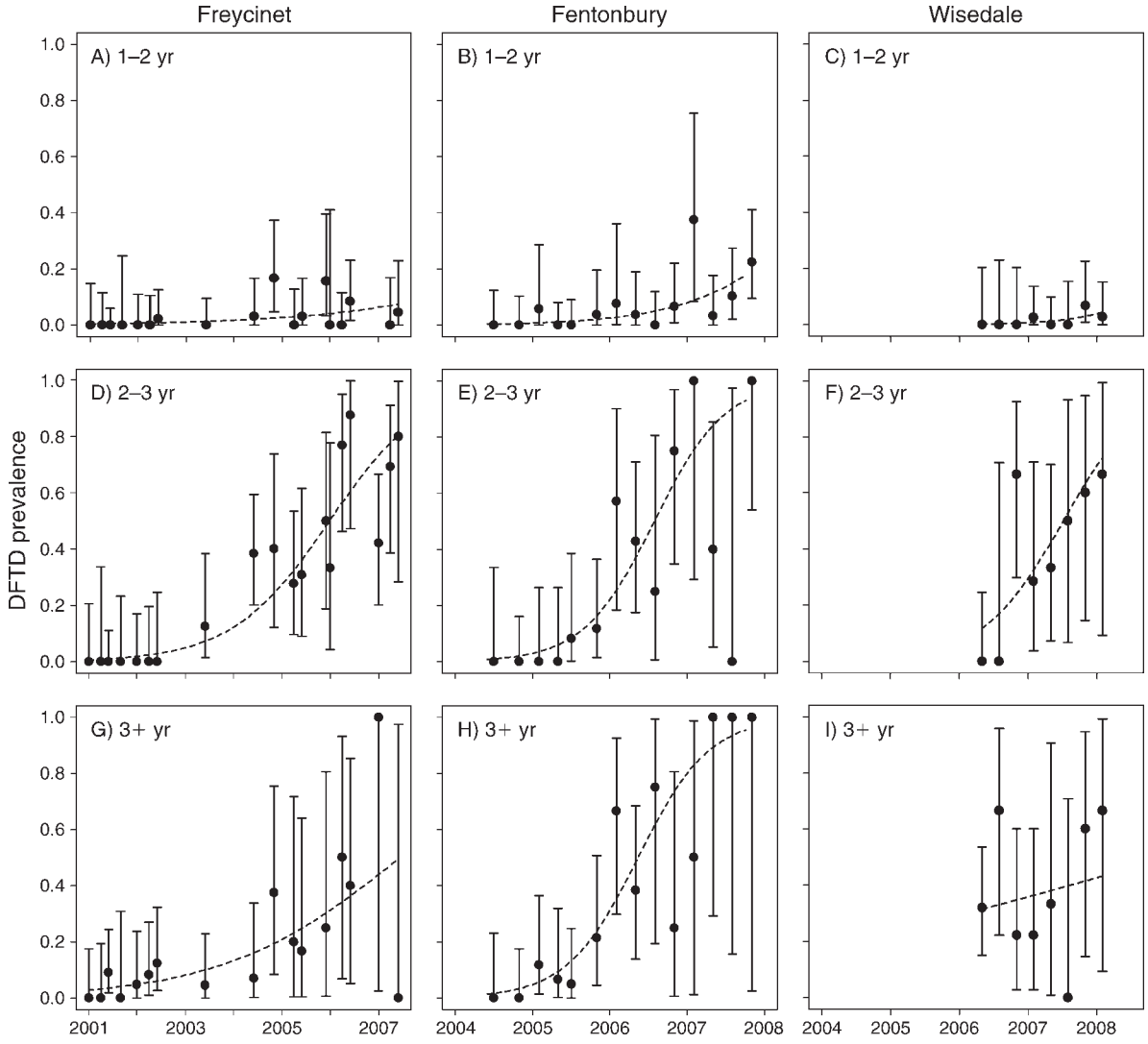

FIG. 2. Prevalence of Tasmanian devil facial tumor disease (DFTD) at three sites: Freycinet (A, D, and G), Fentonbury (B, E, and H), and Wisedale (C, F, and I), monitored from before or close to the onset of disease. (A–C) Tasmanian devils from 1–2 years of age, (D–F) devils 2–3 years of age, (G–I) devils 3+ years of age. Error bars are 95% exact binomial confidence intervals, and the dashed lines represent the best fit of a logistic regression model including age class and trend but without seasonal effects.

and 2–3-year-old devils, for all season–site combinations in which the effects were estimable. Effects are corrected for trend in those sites where it was well supported in the previous analysis (Fentonbury, Wisedale, Freycinet, and Mt William). Effects of season are strongly negative in one-year-olds in autumn (immediately after their presumed birthday), but then appear to decrease in magnitude through the year. There is no evidence of consistent seasonality across sites in two-year-olds.

Impacts on devil population size

At all sites, except Buckland and Wisedale, the estimated devil population size declined in the presence of the disease (Fig. 5, Table 6). There was no evidence that disease prevalence in adult devils declined with population size, as would be expected if transmission were density dependent.

Estimates of R_0

Table 7 shows estimates of R_0 based on the Freycinet and Fentonbury models reported above. Estimates for Freycinet are considerably lower than those at Fentonbury for the same serial interval. The extreme influence of the generation time estimate on R_0 is also evident.

Alternate models of disease transmission

Fig. 6 shows trajectories of devil density and DFTD prevalence predicted from both density-dependent and frequency-dependent transmission for a range of plausible latent periods. With frequency-dependent trans-

TABLE 2. Generalized mixed models of Tasmanian devil facial tumor disease prevalence at three sites, where monitoring commenced close to DFTD arrival.

Model terms	Parameters	ΔAIC_c	Akaike weight, w_i
Freycinet ($N = 54$)			
Ad.juv × trend	5	0.00	0.196
Ad.juv + trend	4	0.40	0.160
Age class × trend	7	0.49	0.153
Ad.juv × (trend + season)	11	0.56	0.147
Ad.juv × trend + summer	6	2.18	0.066
Age class + trend	5	2.48	0.056
Age class × trend + summer	8	2.48	0.056
Ad.juv + trend + summer	5	2.62	0.053
Fentonbury ($N = 42$)			
Ad.juv × trend + summer	6	0.00	0.359
Ad.juv × summer + trend	6	1.24	0.194
Ad.juv + trend + summer	5	1.61	0.161
Ad.juv × (trend + summer)	7	1.89	0.140
Wisedale ($N = 24$)			
Ad.juv + trend	4	0.00	0.479
Ad.juv	3	2.76	0.121
Ad.juv × trend	5	2.87	0.114

Notes: Age class represents three age classes, 1–2-year-olds, 2–3-year-olds, and 3+ year-olds; trend is time in years since disease emergence at the site; season is the four Austral seasons; ad.juv is age class contrasting 1–2-year-olds vs. older animals, and "summer" contrasts summer with the remaining seasons. A "+" represents additive effects, whereas a "×" also includes interactions. Models for each site are shown in order of increasing AIC_c (small-sample corrected Akaike Information Criterion). All possible models including up to two-way interactions were fitted, but only models with a difference in AIC_c from the best model (ΔAIC_c) of <3 are shown. The table also presents the Akaike weight w of these plausible models (calculated relative to all the models fitted for that site). This can be considered as the weight of evidence in support of the model or (loosely) as the probability that the model is the best of those considered for the data (Burnham and Anderson 2004). Also shown is the number of parameters for each model and the sample size N (trip–age class combinations) at each site.

mission, the model predicted the maintenance of high prevalence levels in populations where devil density has declined substantially after several years since disease introduction, consistent with our field observations (Fig. 6). The model predicts devil decline to infinitesimal levels, which would correspond to host extinction in any finite population. The equilibrium prevalence is higher for shorter latent periods. The same model formulated with density-dependent transmission was unable to generate high levels of DFTD prevalence with decreasing population abundance, whatever the latent period, and predicts maintenance of devil numbers at around 40% of the original carrying capacity, with oscillations in host numbers damping more rapidly as the latent period increases. As is predicted by simpler, nonstructured models (de Castro and Bolker 2005), the two transmission modes thus produce very different outcomes, with a stable equilibrium between host and pathogen generated from density-dependent transmission, but with host extinction predicted from frequency-dependent transmission.

TABLE 3. Generalized mixed models comparing increases in prevalence with time in 2–3-year-old Tasmanian devils at Freycinet, Fentonbury, and Wisedale.

Model terms	Parameters	ΔAIC_c	w_i
Site × trend	7	0.00	0.560
Site + trend	5	2.07	0.199
Site × trend + summer	8	3.01	0.124
Site × (summer + trend)	10	4.44	0.061
Site + trend + summer	6	4.74	0.052

DISCUSSION

Prevalence of DFTD is very high in 2–3-year-old individuals at all sites, once disease has been present in the area for several years. Given that the disease is typically fatal within six months of the first appearance of a visible tumor (Hawkins et al. 2006), infection levels in excess of 50% in all 2–3-year-olds present at any given time mean that the disease is having a devastating impact on Tasmanian devil populations. This can be seen from the ongoing population declines at almost all sites when disease is present (see Fig. 5). The lack of significant declines in population size following DFTD introduction at Wisedale and Buckland is likely to be an artifact of the short time-series available for these two sites. Further analysis of trapping data from all sites (Jones et al. 2008) shows a total change in the life history of Tasmanian devils in diseased populations, with an almost complete disappearance of animals older than three years of age.

Particularly concerning is the lack of evidence of any substantial decrease in prevalence in populations such as Mt William, in which the disease has been established for lengthy periods, despite the overall population size being reduced by as much as 90% since 1996 (based on estimates from spotlighting counts [McCallum et al. 2007]). Comparison of Figs. 2 and 3 suggests that prevalence in 2–3-year-olds is at least 50% in populations where the disease is well established, but may reach 80% or more within four years of disease arrival. However, prevalence in 1–2-year-old animals appears to be greater in the populations where disease is established than it is in the populations monitored early after the arrival of the disease.

TABLE 4. Parameter estimates for the most strongly supported model (site × trend) of the prevalence of Tasmanian devil facial tumor disease in 2–3-year-old devils at three sites.

Parameter	Estimate	Lower	Upper
Intercept	−5.879	−8.720	−3.920
Freycinet	0.836	−2.156	3.904
Wisedale	3.057	−0.115	6.616
Trend	2.268	1.450	3.483
Freycinet : trend	−1.254	−2.464	−0.301
Wisedale : trend	−0.413	−2.411	1.692

Notes: Site effects are relative to Fentonbury. Lower and upper 95% confidence bounds were derived from Highest Posterior Density intervals from a Markov chain Monte Carlo sample ($n = 50\,000$).

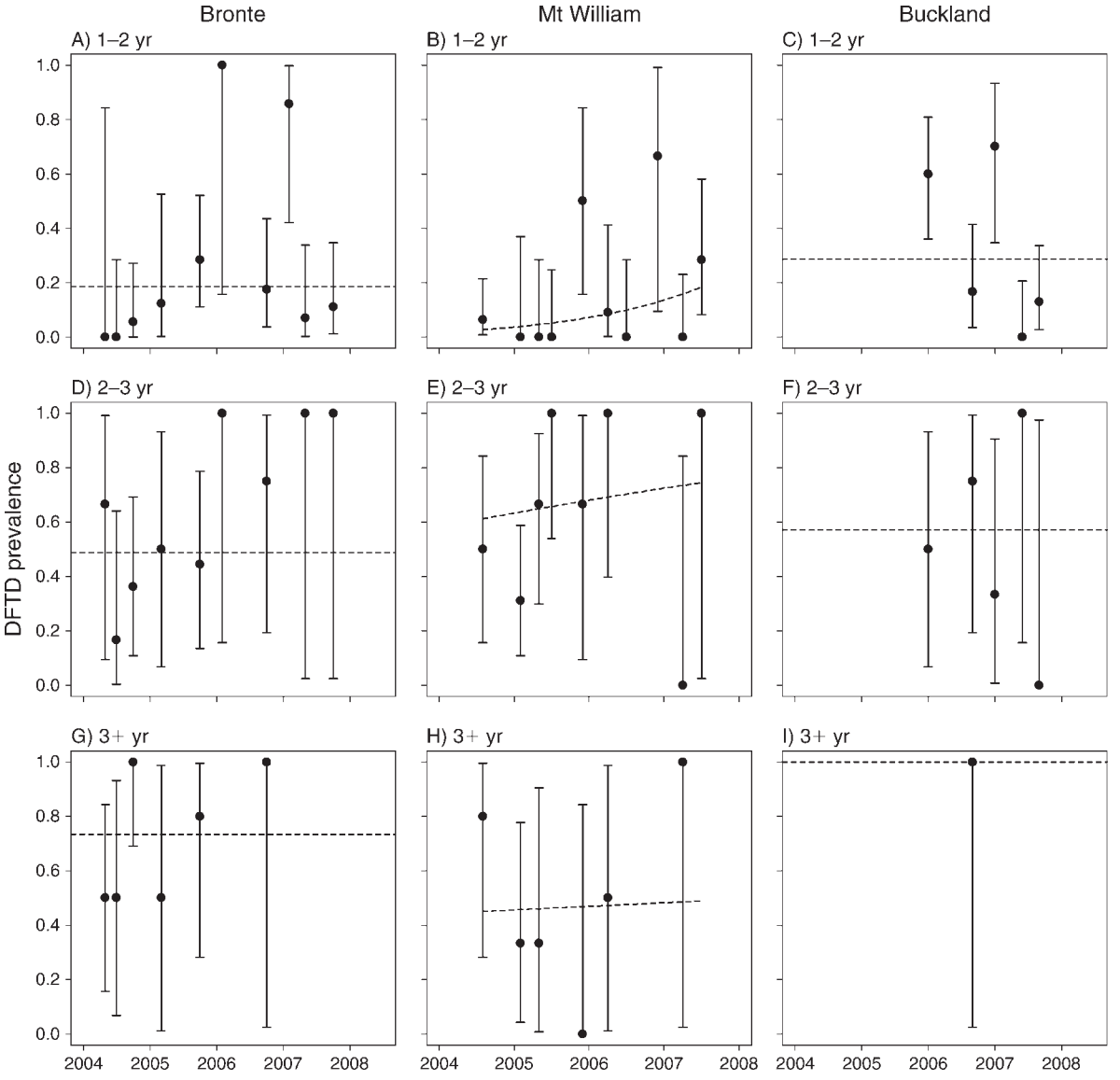

FIG. 3. Prevalence of DFTD at three sites where monitoring began well after disease was established: Bronte (A, D, and G), Mt William (B, E, and H), and Buckland (C, F, and I). (A–C) Tasmanian devils 1–2 years of age, (D–F) devils 2–3 years of age, (G–I) devils 3+ years of age. Error bars are 95% exact binomial confidence intervals. The dashed lines in the Mt William panels represent the best fit of a logistic regression model including age class and trend but without seasonal effects. As there was no evidence of a trend at the other two sites, the dashed lines at the other two sites represent the mean prevalence in that age class across all sampling intervals.

The very high levels of prevalence reached and maintained in 2–3-year-olds indicate that transmission depends weakly, if at all, on host density, and shows no evidence of any threshold population density below which the disease cannot be maintained within the Tasmanian devil population. Our simple age-structured model suggests that the high levels of prevalence maintained even after large population declines are inconsistent with the hypothesis of density-dependent disease transmission, but are more consistent with transmission being dependent on the frequency of infected hosts in the population. The predicted prevalence for frequency-dependent transmission if a 3-month latent period is assumed is close to the observed prevalence. However, the simplified way in which time delays are modeled in Eqs. 2–5 limits the extent to which the quantitative predictions of prevalence from the model can be directly compared with those observed in Figs. 2 and 3. In the absence of a threshold population density for disease maintenance, it is theoretically possible for a host-specific pathogen such as DFTD to lead to host extinction (de Castro and Bolker 2005).

Substantial reductions in population size following disease epidemics may cause density-dependent com-

TABLE 5. Generalized mixed models of Tasmanian Devil facial tumor disease prevalence at three sites, at which disease was well established when monitoring commenced.

Model terms	Parameters	ΔAIC_c	w_i
Mt William ($N = 44$)			
Ad.juv × season + trend	8	0.00	0.346
Ad.juv	3	2.50	0.099
Ad.juv × (trend + season)	9	2.67	0.091
Ad.juv × season	7	2.79	0.086
Bronte Park ($N = 25$)			
Age class + season	7	0.00	0.228
Age class + summer	5	0.84	0.150
Ad.juv + summer	4	1.00	0.138
Age class + summer + trend	6	1.12	0.130
Ad.juv + season	6	1.35	0.116
Ad.juv + summer + trend	5	1.99	0.084
Buckland ($N = 11$)			
Null	2	0.00	0.400
Ad.juv	3	1.48	0.191
Trend	3	1.50	0.189

Notes: As in Table 2, age class represents three age classes, 1–2-year-olds, 2–3-year-olds, and 3+ year-olds; trend is time in years since disease emergence at the site; season is the four Austral seasons; ad.juv is age class contrasting 1–2-year-olds vs. older animals, and "summer" contrasts summer with the remaining seasons. A "+" represents additive effects, whereas a "×" also includes interactions. Models for each site are shown in order of increasing AIC_c (small-sample corrected Akaike Information Criterion). All possible models including up to two-way interactions were fitted, but only models with a difference in AIC_c from the best model (ΔAIC_c) of <3 are shown. At both Bronte Park and Buckland, the data were too sparse for models including interactions with age class and season or summer to be fitted. The table presents the Akaike weight w of these plausible models (calculated relative to all the models fitted for that site). Also shown is the number of parameters for each model and N, the sample size (trip–age class combinations) at each site.

pensatory changes in host population dynamics, mediated via changes to the life history traits of individuals, which can potentially mitigate the population decline (Fowler 1981). In areas where DFTD has become established, there is evidence of increased breeding in females between one and two years of age. (Prior to the disease the majority of female devils began breeding at two years of age [Jones et al. 2008].) Widespread precocial breeding in Tasmanian devils, however, is precluded by physiological and ecological constraints that limit the ability of one-year-olds to breed (Lachish et al. 2009). Since no other compensatory responses to population decline have been observed (Lachish et al. 2009), the observed reproductive compensation will be unlikely to greatly alter a population's trajectory once disease is established. Further, the frequency-dependent transmission described in this paper, together with the observation that bite injuries are particularly prevalent in the mating season (Hamede et al. 2008) suggest that DFTD has some attributes of a sexually transmitted disease. Precocial breeding might therefore be associated with increased disease in young animals. (Compare Figs. 2 and 3 for DFTD prevalence in 1–2-year-olds.)

Transmission is the key process in the dynamics of an infectious disease, but is invariably the most difficult process to parameterize, particularly in an emerging disease of wildlife. DFTD is no exception. In both of the sites from which we have good data from the time of the first appearance of the disease (or very soon thereafter), the disease is initially at higher prevalence in older animals than in younger animals. This would be expected given that there appears to be a substantial incubation period and that bite injuries occur more frequently in adults than in subadults (Hamede et al. 2008). Increased prevalence in older age classes is expected in most diseases that produce persistent infections, simply because older animals have had more time to become infected (Grenfell and Anderson 1985). In the case of DFTD, however, infected animals are removed from the population within ~6 months because the disease is invariably lethal, meaning that the

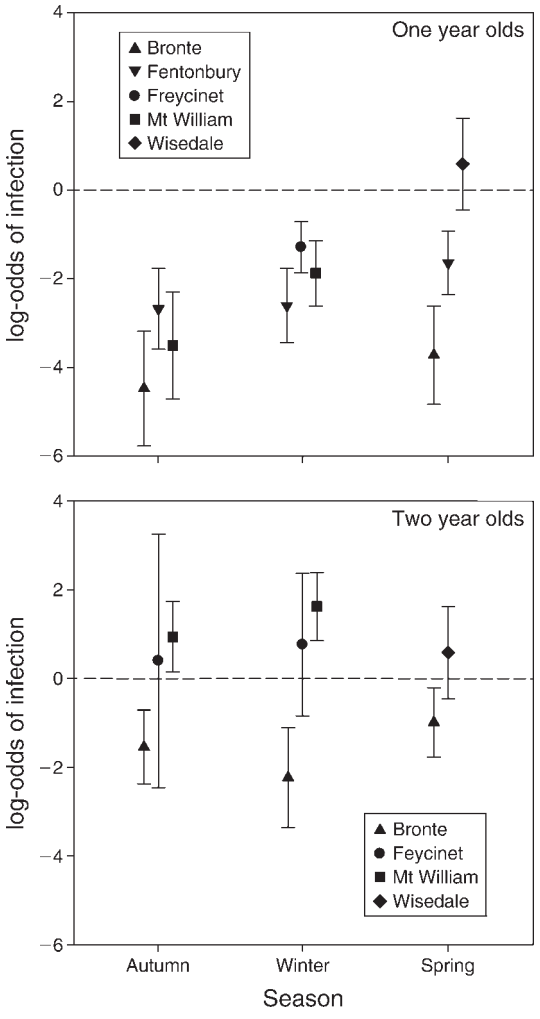

FIG. 4. Estimates of effects of season (relative to summer) on log-odds of infection, with standard errors. Bronte, up-pointing triangles; Fentonbury, down-pointing triangles; Freycinet, circles; Mt William, squares; Wisedale, diamonds.

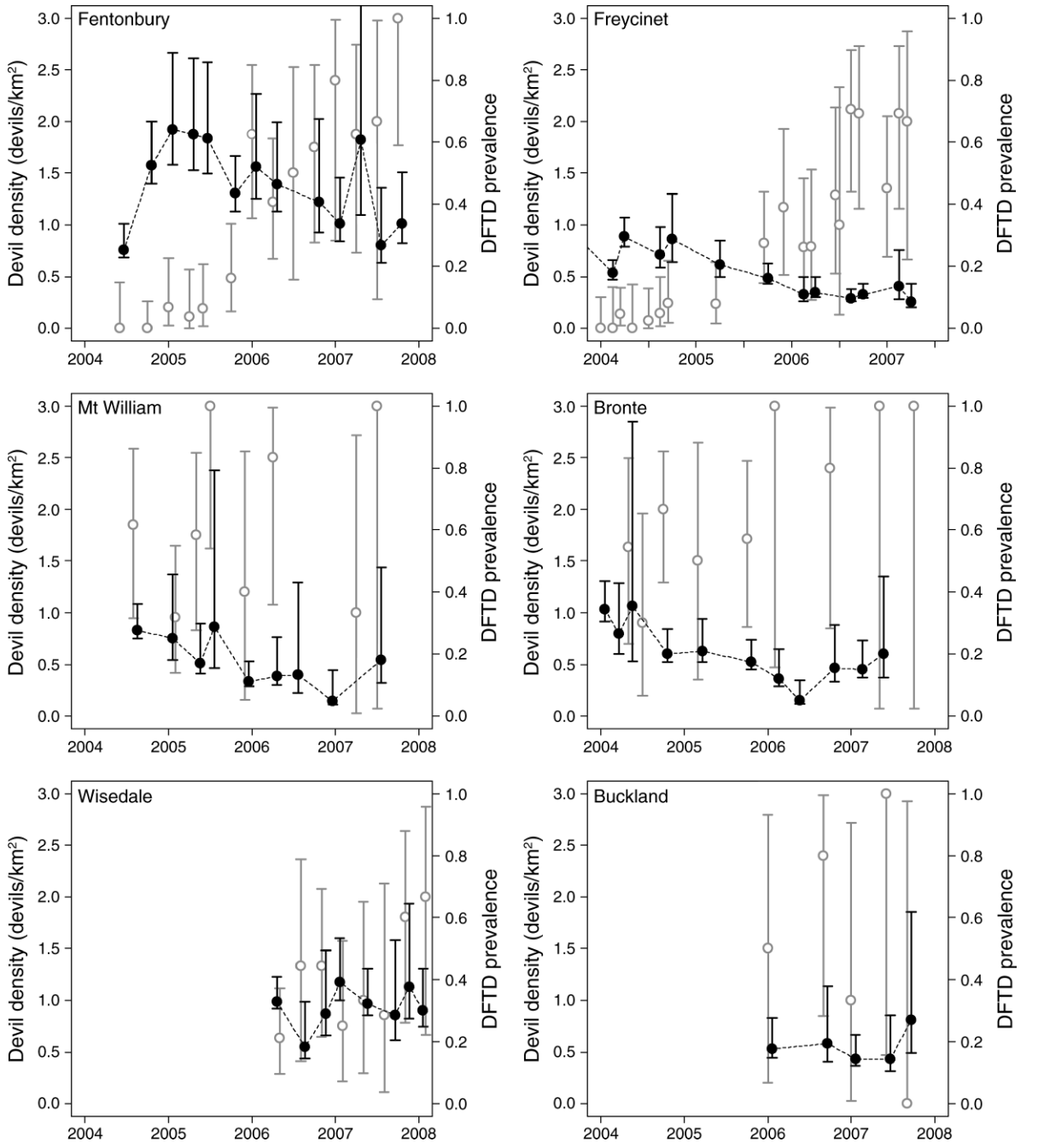

FIG. 5. Estimates of population density (devils/km^2, with 95% confidence intervals; black lines and solid symbols) of Tasmanian devils at the six study sites. The prevalence of DFTD in adult animals (2 years and older; with exact binomial 95% confidence intervals; gray lines and open symbols), is also shown, with scale numbers on the right axis of each panel. Note that data from 2001 are included in the graph for Freycinet.

predicted relationship between age and prevalence is not as straightforward.

While there is evidence of a seasonal pattern in prevalence in one-year-olds at most sites (see Fig. 4), this probably does not represent a seasonal pattern in transmission. Devils are born over a 6-month period, although half of births occur in March (Hesterman 2008). This means that "1–2-year-olds" sampled in autumn have probably just passed their first birthday, and have only been weaned for 3–4 months, whereas 1–2-year-olds sampled in summer are nearly two. The diminishing effect on prevalence of season relative to summer in 1–2-year-olds in Fig. 4 is therefore most likely simply a function of longer potential exposure of

TABLE 6. Trends in Tasmanian devil population density at six sites when disease was present.

Site	df	b	SE(b)	P
Freycinet	10	−0.163	0.0266	0.0001
Fentonbury	9	−0.259	0.0487	0.0004
Wisedale	6	0.010	0.112	0.927
Mt William	7	−0.5511	0.1148	0.0020
Bronte	9	−0.3403	0.1089	0.012
Buckland	3	−0.0865	0.1515	0.608

Note: The trend parameter (b) is shown together with its standard error and significance, derived from a regression of ln(population density) vs. time in years, weighted by the estimated density divided by the square of the width of the confidence interval.

those animals to infection. The lack of a similar pattern in 2–3-year-olds suggests that a seasonal effect is not due to a seasonal change in the force of infection. This is a rather unexpected result. The most likely means of disease transmission is through biting, and there is evidence that, in adults, biting injuries are typically around twice as common during the mating season (March) as in other seasons (Hamede et al. 2008). An increase in disease prevalence would therefore be expected following a lag equal to the average incubation period, particularly as infections progress from first detection to death within six months. One explanation for the lack of a seasonal peak in prevalence is that the incubation period may be variable between individual infections, depending on a range of factors, which might include the number of infected cells transferred, the location of the transfer, and the genotype and immunological status of the infected host. Another factor that may act to distribute any seasonal peak in transmission detectable through prevalence is that in this analysis, early- and late-stage infections are not distinguished.

The estimates of R_0 reported here differ substantially between the two areas from which they were derived, being significantly higher at Fentonbury than at Freycinet (see Table 7). This is to be expected: R_0 is not an intrinsic property of a disease, but depends on the environment in which the disease occurs, and also may depend on population density. The estimated population density at Fentonbury was indeed higher than at Freycinet at the time of disease arrival (see Fig. 5). However, the very high prevalence in adult (2+ years) devils maintained at all sites, despite major declines in density, suggests that transmission depends weakly on population density. A contributor to the higher rate of increase in prevalence with time at Fentonbury compared with Freycinet is likely to be the different physical properties of the two study areas. Freycinet is an extended linear site (~50 km from north to south), and the disease entered the northern part of the study area several years before it spread to the south. The increase in prevalence at Freycinet therefore includes a component of spatial spread through the study site, in addition to increasing prevalence in individuals present at any given location. In contrast, the Fentonbury study site is smaller (~5 km across in any direction), and the increase in the number of individuals infected at that site does not contain a substantial element of spatial spread.

The quantity that can be empirically measured from our data is r_0, which is the increase in prevalence per unit of time, on a logarithmic scale. However, the parameter of key epidemiological interest for management purposes is R_0, the basic reproductive rate, which is measured on a per generation scale. Unfortunately, a complicating factor in this case is that we do not have a good measure of the generation time of the disease, which is the mean time between an individual acquiring infection and transmitting it to another (Svensson 2007). For a given value of r_0, R_0 is greater for a longer generation time. The lack of knowledge of the generation time is responsible for the very large differences in the plausible values for R_0 shown in Table 7. If the lower estimates are closer to the true value, then controlling the disease by vaccination or by the removal of infected animals may well be feasible. For example, developing a vaccine is a potential management strategy (Woods et al. 2007). A standard equation (Anderson and May 1991) for the proportion of individuals p that must be vaccinated in order to eliminate a disease from a population is

$$p = 1 - 1/R_0. \quad (6)$$

TABLE 7. (A) Estimates of r_0 (the initial increase in prevalence per year), derived from generalized mixed models of prevalence in 2–3-year-olds vs. time in years, and (B) estimates of R_0 (the basic reproduction number), derived from Eq. 1 for plausible values of the generation time of the disease (T, in years).

	Freycinet			Fentonbury		
Statistic	2.5%	Estimate	97.5%	2.5%	Estimate	97.5%
A) r_0	0.6257	1.0055	1.2912	1.4663	2.2644	3.1208
B) R_0						
$T = 0.25$	1.169	1.286	1.381	1.443	1.761	2.182
$T = 0.50$	1.367	1.653	1.907	2.082	3.102	4.761
$T = 0.75$	1.599	2.126	2.634	3.003	5.465	10.387
$T = 1.00$	1.870	2.733	3.637	4.333	9.625	22.664

Note: The percentiles shown (2.5%, 97.5%) were derived from Highest Posterior Density intervals for r_0 from a Markov chain Monte Carlo sample ($n = 50\,000$ iterations).

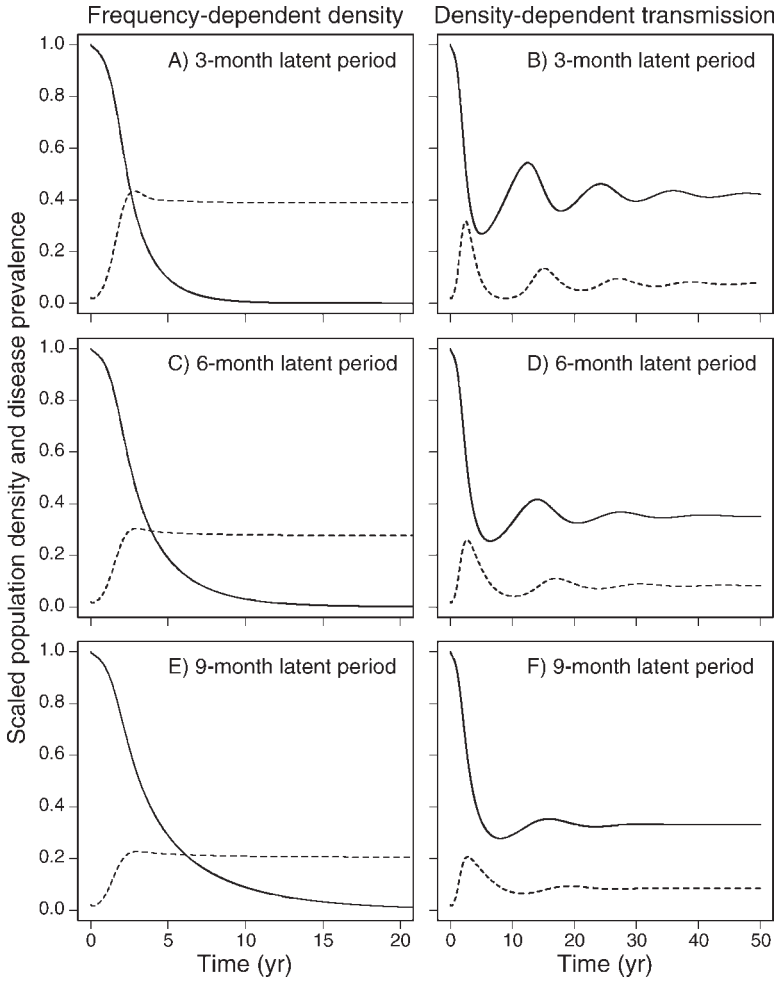

FIG. 6. Solutions of a simple age-structured model of Tasmanian devil–DFTD dynamics, with frequency-dependent disease transmission (A, C, and E) and density-dependent disease transmission (B, D, and F). In each, Tasmanian devil population density through time, scaled relative to the disease-free equilibrium population (solid line) and disease prevalence in animals two years and older (dashed line) are shown. Transmission for both density-dependent and frequency-dependent models was scaled so that the initial increase in prevalence matched that at Fentonbury (2.26 yr^{-1}) and the maximum rate of increase of the devil population, r_{max}, was set at 0.3 yr^{-1}. As the latent period is the most poorly known parameter, results for latent periods of (A, B) 3 months, (C, D) 6 months, and (E, F) 9 months are shown. The mortality rates of susceptible and exposed devils are: 0.211, 0.144, 0.133, 0.146, 0.353 (all yr^{-1}) for age classes 0–1, 1, 2, ..., 4–5, respectively. Those of infected devils are 0.951, 0.946, 0.946, 0.945, 0.956 yr^{-1}, which represents an additional age-independent mortality rate of 93.8% per year.

Given the mean estimated value for R_0 at Freycinet and a generation time of six months, this would suggest that vaccination of only ~30% of the population would be sufficient to eliminate the disease. Tasmanian devils are relatively trappable: mark–recapture models from Freycinet estimate the capture probability over a seven-day field trip of an individual known to be present in the population at 79% (Lachish et al. 2007). Even allowing for the possibility that some proportion of the population might be untrappable, this high recapture probability suggests that vaccination of 30% of the population might be achievable even if a vaccine needed to be delivered by injection. However, if the estimated value of r_0 at Fentonbury is closer to the typical value and the generation time is one year, then in excess of 80% of the population would need to be vaccinated, which would be more difficult to achieve.

The possibility of controlling the disease by selective culling of symptomatic infected animals is under active experimental investigation (Jones et al. 2007). Whereas vaccination aims to limit transmission by reducing the susceptible host population, selective culling aims to limit transmission by reducing the infected host population. The proportion of infected hosts that must be removed per unit of time to eliminate disease would not have a simple form like Eq. 6, because culling, unlike vaccination, will change overall host density. Nevertheless, this proportion will be a function of R_0, as disease elimination by whatever means requires driving R_0 to below 1.

Clearly, a priority for future research must be to produce estimates of the incubation period for the disease to enable more accurate estimation of R_0. However, this is not a straightforward exercise. Even in human disease epidemics when the onset of each individual case is known, estimating the serial interval is a substantial statistical challenge (White and Pagano 2008). Limited evidence from a very small number of laboratory transmission trials (Pyecroft et al. 2007) suggests that the incubation period may depend strongly on the mode of transmission. Experimentally duplicating contacts that are typical for devils interacting in the field is not easy, although confining an infected devil together with susceptible individuals for a short period of time might be possible. Another approach would be to bring animals without disease signs from a diseased area into captivity and then to monitor the time taken until clinical disease was observable. The longest periods measured would produce an estimate of the incubation period that was biased low, but would be an improvement on the anecdotal information currently available. A further possibility might be to measure tumor growth through time in a number of individuals and then attempt to extrapolate backwards to estimate the time of first infection. This would assume that tumor growth was monophasic.

Tasmanian devil facial tumor disease is, to our knowledge, the first case of a host-specific disease threatening to cause the extinction of its host. Detecting the nature of the relationship between transmission and host density is difficult in free-ranging populations (Begon et al. 2003, Rachowicz and Briggs 2007, Davis et al. 2008). The extensive field data analyzed in this study strongly suggest that DFTD transmission cannot be adequately represented by a simple density-dependent model, and therefore that extinction of the Tasmanian devil from this host-specific pathogen is a real possibility. This worrying prognosis for devil populations in the absence of intervention is central to determining appropriate management strategies and indicates the need for substantial investment in establishing disease-free insurance populations, so that disease-free animals can be reintroduced following disease-induced extinction (Jones et al. 2007).

This case study emphasizes the importance of rapidly implementing monitoring programs, should an emerging pathogen be detected in any wildlife species. It is principally from the sites monitored since the first arrival of the disease that we have been able to estimate parameters associated with disease transmission. This information has been critical in determining the prognosis for the species and in justifying the very substantial investment that has been made by Australian governments in its conservation.

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